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CORRIGENDA

Page 212, second paragraph, last line, after "equally bad" insert:
"and snails are common during the rainy season,"

Page 215, Legends to Figs. 1—4, the magnifications should be altered to read:
Fig. 1, ×115; Fig. 2, ×230; Fig. 3, ×230; Fig. 4, ×485.

VOLUME XII DECEMBER, 1920 No. 4

ON SOME NEW HAEMOGREGARINES FROM BRITISH EAST AFRICA.

BY CECIL A. HOARE, B.Sc.

(*From the Zoological Laboratory of the University of Petrograd and the National Institute for Medical Research, London.*)

(With Plate XVIII.)

AT the beginning of 1917 Professor V. Dogiel kindly handed over to me, for scientific treatment, a series of blood-films taken from different Amphibia, Reptilia, and Mammalia by the Expedition of Professors V. Dogiel and I. Sokolov to British East Africa in 1914. The films were examined by me for blood parasites, with the result that, of 27 animals, two different species of *Bufo* and two snakes proved to be infected. The blood parasites were represented by haemogregarines exclusively. The blood-films had been fixed on cover-slips by the dry method, and preserved in that condition for nearly three years. Altogether, I had at my disposal only 24 slips with blood-films taken from the peripheral blood of the infected animals.

For staining, I adopted the Romanovsky-Giemsa solution, Delafield's haematoxylin combined with aqueous eosin, and Pappenheim's combination of Jenner's stain and the Romanovsky-Giemsa solution. On account of the length of time during which the preparations had been preserved in a dry condition, the staining could not have been expected to produce quite favourable results. Thus, the stroma of the erythrocytes when stained with Giemsa's solution assumed a light blue, or greenish colour, instead of pink; the structure of the protoplasm in the parasites was not distinctly exhibited, their nuclei also being often hardly visible.

As regards the hosts of the parasites, it is only in one case that the specific name can be stated, namely in a Puff-adder, *Bitis gabonica*, whereas the second snake, and the two representatives of the Anura in which parasites have been found, will be defined only after the preparations of these animals are received from Alexandria, where the Expedition was compelled to leave the bulky materials collected, owing to the outbreak of war[1]. In due time it is hoped that the hosts will be named in a supplementary note to this paper.

Notwithstanding all these unfavourable conditions, the material presented some interesting data.

[1] V. Dogiel and I. Sokolov. The route and brief description of the travel. *Sci. Res. Zool. Exped. to British East Africa and Uganda in 1914*, Vol. I, Petrograd, 1916.

This work was completed in 1917 in Petrograd, but could not be published in Russia on account of conditions that resulted from the revolution. I am indebted to Professor V. Dogiel for placing at my disposal this material, and for his assistance in the treatment of it. On my arrival in England in May 1920, I had to revise this paper and bring it up to date, since we were altogether out of touch with recent scientific literature in Russia. I avail myself of the opportunity to express my warmest thanks to Professor Clifford Dobell, who has given me valuable advice in my work and provided me with all the necessary literature and accommodation at the National Institute for Medical Research, London. I am further indebted to the Medical Research Council for a grant which enabled me to proceed with this work.

I. HAEMOGREGARINES FROM SNAKES.

Host: *Bitis gabonica* (labelled *Snake No.* 4), from Mabira, June 6th, 1914.

The parasites are encountered within the blood corpuscles exclusively. The degree of infection of the latter is not great. The haemogregarine occupies not more than three-quarters of the length of the host-cell, which remains unaltered both as regards form and size. No influence of the parasite on the structure of the nucleus of the erythrocyte was observed; the nucleus is only dislocated, as is usual in haemogregarinosis.

In form this haemogregarine resembles the generic type represented by *Haemogregarina stepanowi* from a tortoise, *Emys orbicularis* (Reichenow, 1910). The body of our form is more or less bean-shaped, one end being somewhat broader than the other (Plate XVIII, figs. 2, 3). The concave side of the body, in most cases, corresponds to the convexity of the nucleus of the erythrocyte, but frequently this side is turned to the periphery of the blood corpuscle. Minchin (1907) believes that the bean-shaped form of the intracorpuscular parasite is due to its adaptation to the space limited, on one hand, by the convexity of the nucleus of the erythrocyte and, on the other hand, by the curvature of the margin of the corpuscle, and explains the reverse position of the parasite by its active movements preceding the abandonment of the corpuscle. Reichenow (1910), however, disputes this point of view and holds that the bent form of the parasite is due, not to the influence of the nucleus of the host cell, but to the structure of its protoplasm. The latter opinion is perhaps correct, as it may be frequently observed that the nucleus of the erythrocyte is displaced to one of the ends of the parasite, the concavity of which, nevertheless, corresponds to the convexity of the host nucleus.

The protoplasm of the haemogregarine is uniformly granulated, no special inclusions being visible in it. The nucleus is centrally located, occupying about one-quarter of the body length. The parasite is surrounded by a light area. The chromatin substance of the nucleus seems to be arranged in the shape of trabeculae or a network, and sometimes assumes the aspect of a glomerulus resembling a stage of division described by Prowazek (1907) in *Haemogregarina platydactyli*. Such a picture of the nucleus shows that we are

perhaps dealing with a ripe trophozoite or schizont, in which the first indications of the asexual process of multiplication are present. The average measurements of this form are 14μ in length, 6μ in breadth.

Besides this form, sometimes a second is encountered (Plate XVIII, fig. 1), which is more slender, with a smaller nucleus of more compact structure. One end of this form is always bent over in the shape of a small tail. In this form also the light surrounding rim[1] is more sharply outlined, and it is possible that it represents an earlier stage.

In form and size the haemogregarine resembles that described by Dutton, Todd and Tobey (1907), also from a Puff-adder. However, the briefness of their description, and the drawings, do not establish the identity of these two forms. Plimmer (1912) also mentions some haemogregarines found by him in *Bitis arietans*, but his description is still briefer; he characterizes them by only three words "medium, host-cell unaltered." Similar forms were described by Minchin (1910) from a snake in Uganda, but the host is unrecorded. He also examined the blood of a Puff-adder, but with negative results.

As regards the classification of the haemogregarines from snakes, Lutz (1901) considered it possible to unite them all in one species—*Drepanidium serpentium*; most authors, however, follow the principle set forth already by Simond (1901), according to which a definite species of haemogregarines is peculiar to each host species. Dobell (1908) adopts the same view for practical reasons. On the other hand, Sambon (1908) regards the haemogregarines of allied host-species as "host-varieties" or "host-races," corresponding to the "geographical races" of certain free-living animals. At present, however, the haemogregarines are still so inadequately worked out, and so few complete cycles of development are known, that it is impossible to adopt any of these views unreservedly. For practical reasons, Simond's view may be adopted provisionally.

Because neither Dutton, Todd and Tobey, nor Plimmer gave specific names to the haemogregarines found by them, and, because of the opinion just expressed by me, I propose to name the parasite herein described as *Haemogregarina dogieli*, in honour of Professor V. Dogiel.

Host: labelled *Snake No.* 5 ("green tree snake"), from Mabira, June 19, 1914.

In this host the parasites are also intracorpuscular exclusively (Plate XVIII, figs. 5, 6, 7). The erythrocytes are infected in a very slight degree. The haemogregarines usually attain the length of the host cell, or are somewhat longer, slightly bending with their ends inwards. The influence of the parasitism on the host cells is distinctly visible. In my preparations nearly all the erythrocytes infected are diminished in size; as compared with the normal blood corpuscles, their cytoplasm stains very feebly, and, in some cases, there seems to be only a shadow of it visible (dehaemoglobinization). The nucleus

[1] The subject of "light areas," "rims," or capsules in general, is discussed at the end of this paper.

is, on the contrary, in nearly all cases hypertrophied and elongated parallel to the long axis of the parasite (cf. Fig. 4, and Figs. 5, 6, 7), whilst the interior structure of the nucleus loses its normal aspect. The regular rounded lumps of chromatin are no longer visible, and the chromatin mass appears to be entangled in irregular accumulations and strands. In some places vacuoles are visible (Fig. 5). In a word, indications of karyolysis are present.

Possibly in this case the diminution in the size of the erythrocyte presents already a secondary stage in the degeneration of the corpuscle, as this process usually begins with a hypertrophy of the cell and nucleus, succeeded by a diminution and shrinkage of the cell and fragmentation of its nucleus.

The haemogregarine described is disposed parallel to the long axis of the host cell, sometimes closely adjacent to the nucleus of the latter (Fig. 5). The body of the parasite is elongated, slender, never bent over on itself, only the ends may sometimes be slightly bent inwards (Fig. 5). Measurements: length 15 to 16μ, breadth 2·25μ; size of normal erythrocyte 15·5μ × 11μ.

The protoplasm stained very feebly in my preparations; nevertheless, it is possible to trace a slight granulation in it. The nucleus is elongated (3μ long), more or less centrally located.

In general appearance, form of the nucleus, and action on the host cell, our parasite resembles *Haemogregarina* (*Karyolysus*) *crotali* from *Crotalus confluentus* (Sambon, 1909), but it still more closely resembles one of the stages of *Karyolysus gracilis* described by Wenyon (1908) from a lizard—*Mabuia quinquetaeniata*. Like ours, this form presents the only stage found in the peripheral blood, whereas all the other stages of the asexual cycle take place in the internal organs (liver cells). Wenyon, and subsequently Reichenow (1912), regarded this form as a gametocyte (as yet sexually undifferentiated).

Taking into account the pathological action of our parasite on the blood corpuscle infected by it, and its likeness to the representative of the genus *Karyolysus* mentioned above, it is possible to refer our form to the same genus. Although at present we are provided with a description of the complete life-cycle of one representative of this genus—*Karyolysus lacertarum* (Reichenow, 1912), which affords us an exact criterion for distinguishing between the genera *Haemogregarina* and *Karyolysus* independently of their pathological rôle—the old classification also remains in force for parasites in which the complete life history has not yet been studied. According to this classification, to the genus *Haemogregarina* are referred all forms which do not do any injury to the nucleus of the host cell, whereas all those that produce such an effect are referred to the genus *Karyolysus* (Reichenow, 1912; Doflein, 1916). Notwithstanding the close resemblance between our form and *K. gracilis*, I prefer to leave the question regarding the species of our parasite open, on account of the presence of only one stage in my preparations.

2. HAEMOGREGARINES FROM AMPHIBIA.

Host: labelled *Bufo No.* 9, from Molo, July 3, 1914.

In the blood of this host the haemogregarines are encountered in large numbers, both intracellularly and free in the blood plasma. The influence of the parasite on the erythrocyte is considerable: both the cell-body and the nucleus undergo alterations. We shall return to these changes below.

The haemogregarines are represented by a whole series of forms, evidently representing stages of the asexual cycle of multiplication. For the sake of convenience, we may divide the forms met with here into the four following types:

Type 1. Elongated slender form, thickened and rounded at one end and gradually tapering to a tail at the other (Figs. 9, 10, 11). The measurements of these forms are 25μ in length and 3μ in breadth. The nucleus is elongated, situated nearer to the blunt end, of rather compact structure. The protoplasm is granulated. These forms are encountered sometimes free in the plasma (Fig. 9) and sometimes in the act of entering or leaving the erythrocyte (Figs. 10, 11). According to the data of some authors (Stebbins, 1905) the blunt end of the parasite should be regarded as the anterior end, as it is this end that is directed forwards during progression of the parasite. Therefore Fig. 10 represents the parasite penetrating into the corpuscle, whilst Fig. 11 shows its emergence. Probably the temporary invasion of the blood corpuscle by the parasite does not remain without effect on the former. This influence is visible on comparing Fig. 8 with Fig. 11. Pictures similar to those described here from fixed preparations were observed by Stebbins (1905) in *Rana clamata* in the living state. This author states that the parasite swims in the blood plasma and "is able to enter and leave the blood corpuscles with the greatest ease and rapidity, and always mutilates the corpuscles badly in so doing." Dobell (1910) also found free forms of *H. berestneffi* (from *Rana tigrina*) which were actively motile. He "observed small forms enter red corpuscles. They did this by boring directly into the corpuscle....Occasionally, the animal, after reaching the inside of the corpuscle, rested for a few minutes and then wriggled its way out again into the blood plasma." The same observer tells me that he saw similar phenomena in the case of the haemogregarines of the rat-snake (*Zamenis mucosus*). Probably the same takes place in our case as well. This is also demonstrated by the fact that fragments of erythrocytes and their free nuclei are frequently encountered amongst intact blood corpuscles.

It should be noticed here that many authors, *e.g.* Flu (1910), Reichenow (1910), Sambon (1908), Shortt (1917), assert that the extracorpuscular state of the parasite is due to abnormal conditions arising on account of the blood being exposed to the air for some time, whereas normally, and when immediately fixed, all the haemogregarines remain intracorpuscular. This view is categorically refuted by Seidelin (1911), who had "seen them free in pre-

parations which were taken from the living animal and immediately fixed," and by Schubotz (1913), who observed very numerous free forms of *H. pettiti* (from *Crocodilus niloticus*) in the blood from peripheral vessels and from internal organs (liver and spleen). In fresh preparations sealed with vaseline this author kept the free forms living for hours. To this question I cannot give a definite answer based on my own material, but judging from pictures like Figs. 10 and 11, and from the complete absence of these forms within the blood corpuscles, it is easier to agree with Seidelin. The phenomena observed by Stebbins and Dobell give further confirmation of it.

Type 2 is represented by intracorpuscular forms of different size; one end of their body is much thickened, the other terminates in a short point, the body being bent over in different degrees (Figs. 12, 13, 14). The nucleus is of the usual vesicular structure, and the protoplasm is granulated, the granulation being coarser than in the preceding form. Sometimes the protoplasm contains dark staining lumps, probably of the so-called volutin (Figs. 13, 14). The blood corpuscle infected by this form is hypertrophied (Fig. 13) and its nucleus undergoes degeneration (Fig. 12).

The most numerous are forms belonging to

Type 3. All of these are intracorpuscular, the body is of an irregular bean-shaped form, measuring $18{\cdot}8\,\mu \times 7{\cdot}8\mu$. The nucleus of the parasite is disposed nearer to one of the ends of the body, the protoplasm is granular, and probably sometimes contains volutin granules (Fig. 15). This form is, moreover, characterized by the constant presence of a kind of dark cap situated on one end of the body (Figs. 15, 16). This formation evidently presents the frequently described accumulation of excretions of the parasite (Dutton, Todd, and Tobey, 1907; Stevenson, 1911; MacFie, 1914, and others).

The influence of this form of the parasite on the cell harbouring it is expressed in a general shrinkage of the erythrocyte, the body of which is ultimately reduced to the condition of a thin membrane enclosing the parasite. At the same time the nucleus of the erythrocyte degenerates and often undergoes fragmentation, breaking up into two parts (Fig. 16), as has been already frequently described by other authors (Stebbins, 1905; Plimmer, 1912; Conor, 1912; Reichenow, 1912; Shortt, 1917). This form resembles the haemogregarine described by MacFie (1914) from African toads, and, in some respects, that described by Shortt from *Bufo melanostictus*.

In all the types described above, the parasite is surrounded by a distinct light rim.

To *type* 4 belong parasites whose body is club-shaped, or of irregular form, from 19μ to 34μ long, characterized by a large nucleus of round-oval form and vesicular structure (Figs. 17, 18). All forms of this type were encountered free in the plasma and only in one case a parasite was found in the act of leaving the host cell, in which the remains of the abandoned capsule are visible (Fig. 17). Similar forms were described by Dutton, Todd, and Tobey (1907), Flu (1910), and others.

As regards the mutual relationship between all the forms described above, we can, of course, only make such suppositions as are based on cycles of development already studied.

The forms of *type* 1 (Figs. 9, 10, 11) doubtless represent the young stages of the parasite just beginning its cycle of development. Probably they are merozoites and pass through a certain period of free life, in the course of which they penetrate into the erythrocytes for a short time (sometimes pictures are seen in which only the middle portion of the parasite lies in the blood corpuscle, whilst its ends are free), as was stated by Stebbins (1905), Dobell (1910), Neresheimer (1909), and others. This stage is probably succeeded by an intracorpuscular stage, during which the parasites grow and bend over, adapting themselves to the limited space within the erythrocyte (Figs. 12, 13). In this period there appear in the protoplasm of the parasite the characteristic granules of volutin.

The *volutin* probably represents reserve material formed in the protoplasm and used up for the formation of nuclear substances during the maturation of the nucleus in the period of multiplication. The appearance of volutin in the protoplasm indicates the beginning of the processes of reproduction (Reichenow, 1910; Doflein, 1916).

Taking into consideration the circumstances stated above, we may suppose that the forms described as *type* 2 (Figs. 12, 13) represent adult, ripe trophozoites. The form represented in Fig. 14 probably shows the stage at which one of the limbs of the body is retracted. Such a process usually precedes the formation of the schizont (Reichenow, 1910).

The forms of *type* 3 are encountered in the films in predominant and very large numbers. It is to be supposed that after complete reduction of the "tail" the form just described (Fig. 14) passes to this stage (Fig. 15); as is also confirmed by the resemblance in the appearance of their nuclei (the structure of the latter is hardly discernible in these stages).

The succeeding stages of development evidently take place in the internal organs.

The forms represented in Figs. 17 and 18 are encountered only in single cases. It is possible that they represent the intermediate stages between the initial (Fig. 15) and final stages of the process of schizogony.

In the forms of *type* 4 (Figs. 17 and 18) a gradual increase in the size of the cell, and a swelling of the nucleus, are visible.

Of course, all the considerations set forth here are hypothetical, as it is impossible to form a definite judgment regarding the successive phases of a complex cycle of development based on separate forms from several blood films.

As has been mentioned, some of our forms resemble, in general features, the parasites described by Dutton, Todd, and Tobey, MacFie, Stebbins, Stevenson, and Shortt. This likeness is, however, always limited to some single stage, whereas the other forms differ sharply from our parasites. More-

over, in most cases it is necessary to compare my findings with drawings alone, as the descriptions are always too short.

One of the forms that most closely resembles ours is *H. nucleobisecans* from *Bufo melanostictus* (Shortt, 1917). This form differs from ours in the presence of small forms, and in its free forms being much larger and of a different shape. *H. nucleobisecans* seems to affect the host nucleus in the same way as ours, splitting it into two in some cases. According to Shortt "this is brought about by the pressure of the concave border of the capsule of the parasite upon the adjacent border of the host-cell nucleus." In this case the fragmentation of the nucleus seems to be purely mechanical, differing in that respect from the fragmentation of the host nucleus seen in the genus *Karyolysus*.

If this be the case in our parasite, it is difficult to decide to which genus it should be referred in the absence of any sexual stages.

For the sake of registration, I propose to refer the parasite, provisionally, to the genus *Haemogregarina* and name it *Haemogregarina moloensis* nov. spec.

Host: labelled *Bufo No.* 8, from Molo, July 3, 1914.

The blood corpuscles of this animal were strongly infected with haemogregarines. The host cell does not seem to undergo any special alterations; the enlargement observed is to be ascribed to mechanical causes, being due to the expansion of the growing parasite. In some cases, however, the nucleus of the erythrocyte is seen to be broken up into two parts.

The haemogregarines occurring in the blood of this host are represented by several forms.

1. The predominant form of the parasite is a large haemogregarine of irregular oval or bean shape, in most cases not bent on itself (Figs. 24, 25), but sometimes with a distinct second limb (Fig. 22), or traces of the fusion of both limbs (Fig. 23). The average measurements of these forms are 17·5μ by 11μ. Their internal structure will be described below.

2. Besides these forms, in much fewer numbers, are encountered comparatively small parasites of pretty regular spindle or oval shape (Fig. 19), measuring 14μ by 4·7μ.

3. There are also encountered somewhat larger parasites in which one end is dilated, and the other gradually attenuates in the form of a pointed tail (Fig. 20). This extremity is usually turned to the side, but not bent over.

4. And lastly there occurs another form, in general resembling the one just described, but more massive and with the tail bent over the body (Fig. 21).

The protoplasm of the last three forms appears to be finely granulated. In the first of them (Fig. 19) vacuoles are visible at both poles (possibly artifacts). The nucleus is usually disposed nearer to one of the extremities of the body. In the second form (Fig. 20) the nuclear chromatin is arranged in rather compact lumps, whilst in the third form (Fig. 21) the nucleus occupies a larger space and its chromatin is arranged more loosely.

The structure of the body of the main form (Figs. 22, 23, 24, 25) differs considerably from that of the preceding. The protoplasm of these forms has a distinct alveolar structure and contains some kind of indefinite accumulations. The nucleus of these forms has lost its definite shape and appears in the form of minute chromatin granules disposed in different ways, but chiefly in the form of a transverse band.

A similar alveolar structure of the protoplasm was described by Nöller (1912), Reichenow (1910), Schubotz (1913), and others, in schizonts, and the arrangement of the nuclear elements in the form of a transverse band is found in parasites described by Langmann (1899), Billet (1907), and Flu (1910), also in the period of their maturation. Both these data and the configuration of the present form, as well as its dimensions, perhaps indicate that we are dealing with a full grown schizont.

As regards the mutual relations between the forms described, they present a fairly complete picture of gradual growth from the young parasite to the stage of schizont.

Form 2 (Fig. 19) indubitably presents a merozoite which had only recently penetrated into the blood corpuscle. It is exactly such a form that is described as typical for the merozoite by those authors who had the opportunity of studying the complete cycle of development of haemogregarines (Reichenow, 1910, 1912, and others). By degrees, as it grows, this form begins to elongate (Fig. 20), thicken and bend over with one end (Fig. 21); this end at length reaches the opposite extremity (Fig. 22), and the parasite assumes the shape of a U. From this moment begins the alteration in the structure of the protoplasm and the nucleus of the parasite pointing to the phenomena of multiplication. In this period both limbs begin to fuse together (Fig. 23), and, finally, the massive oval form (Figs. 24, 25) characteristic of the schizont is produced.

It is interesting to mark certain peculiarities in the disappearance of one of the limbs of the doubled parasite, usually preceding the formation of the mature schizont. This process may be effected in two ways:

1. The tail gradually shortens and appears to be drawn into the main portion of the body. This course was described by Reichenow (1910) in *H. stepanowi*, and also takes place in the haemogregarine described by me above (Fig. 14).

2. The tail bends over and grows to the length of the main portion of the body, and then both limbs fuse along the whole line of their contact. Such a course was described by Simond (1901), and Woodcock (1912). This phenomenon is also observed in the parasite just described (cp. Figs. 20, 21, 22, 23, 24).

In conclusion I shall dwell briefly upon a disputed structure common to nearly all haemogregarines, this structure having been termed by various authors "the light area" or "rim," "membrane," "cyst," and "capsule." With regard to this element various opinions have been expressed.

In most cases it is altogether ignored, or the author barely mentions that the parasite is enclosed in a capsule, without any further definition or description.

With regard to the nature of this structure the opinions of authors differ. Some (Langmann, 1899; Laveran and Pettit, 1911) regard the light area surrounding the parasite as an artificial product, due to unequal contraction of the protoplasm of the parasite and host cell under the action of fixing agents, resulting in detachment of the parasite from the cytoplasm of the erythrocyte; hence the empty space which is absent in normal ones.

The majority of authors (Prowazek, 1907; Dutton, Todd and Tobey, 1907; Dobell, 1908; Sambon, 1908; Robertson, 1908, 1910; Seidelin, 1911; Stevenson, 1911; Conor, 1912; Reichenow, 1912; Schubotz, 1913; Sergent, 1918, and others), however, state definitely that the parasite is surrounded by a special membrane or capsule. Sambon (1908) says:

"The examination of numerous species of haemogregarines...has convinced me that all the endocorpuscular forms, save the very earliest, are enclosed within a shell or capsule produced by the parasite itself, either by means of a special secretion or by a process of ecdysis, the haemogregarine shrinking from its detached former skin, as do certain flies in the formation of their puparium. The capsule varies much in size, shape, thickness, transparency and other particulars according to the species of haemogregarine to which it belongs, as well as to the stage of development....The presence of a capsule may be evidenced by a number of indications. Thus, in preparing films for microscopical examination, some of the haemogregarine-infested blood cells may be disrupted; the parasites remain closely doubled up, being evidently confined by a capsule, to the exterior of which fragments of the host cell nucleus may be seen adhering....Sometimes we may detect a second inner shell produced by a subsequent ecdysis. This inner membrane may also show deeply staining granules. After the escape of the parasite the remains of the broken capsule may be seen either still within the host cell or free in the liquor sanguinis....The capsule appears to burst along definite lines of cleavage.... Two of these lines are to be seen, one at each end of the capsule....After bursting open the two halves of the capsule roll up after the fashion of the mature seed pods of certain leguminous plants."

Similar statements are made by other authors. Thus, Prowazek also found "um den in einer Höhlung der Zelle ruhenden Parasiten...eine rotfärbbare Niederschlagsmembran." Dutton, Todd and Tobey encountered in the blood of snakes and amphibia curled remains of the capsules in the form of "rods" lying in pairs. According to Dobell, Robertson and Sergent the capsule is not only distinctly visible in intracorpuscular forms, but may frequently be encountered either free in the plasma, or in the corpuscles abandoned by the parasites. Seidelin also observed delicate red lines at the extremities of the capsule (Sambon's "lines of cleavage"), and Stevenson stated that the "cyst wall" was double at the end at which the parasite is bent, the space between

the layers being filled up with a dark substance (cp. Fig. 15). Schubotz, on the other hand, declares that in the parasites described by him (from a turtle, *Cycloderma aubryi*) the capsule never appears in the form of a double-contoured membrane.

The two aspects of the question find a compromise in the views expressed by Woodcock (1912) and Shortt (1917). According to these authors, the empty space around the parasites is due to shrinkage, the parasites, however, being surrounded by a distinct thin sheath which envelops their body closely.

My preparations provide no data on which I could base authoritative conclusions. However, it is difficult to interpret the picture presented in Fig. 17 as "shrinkage" of the parasite, and I am inclined to acknowledge the correctness of the second supposition, with the amendments made by Woodcock and Shortt. The question regarding the origin of the capsule—whether it presents the product of secretion of the parasite itself, as Sambon and Woodcock think, or is formed by modification of the adjoining portion of the cytoplasm of the erythrocyte as a reaction against the influence of the parasite—remains open, although the former supposition seems to be more probable.

APPENDIX.

In several blood films from *Bufo No.* 8 and *Bufo No.* 9 were encountered bodies (Figs. 26, 27) which differ so markedly from all the blood parasites hitherto known that I suspect them to have found their way into the blood or preparations by accident, the more so, as they were found in different hosts with distinct parasites.

These bodies are spindle shaped, measuring $7{\cdot}8\mu$ by $1{\cdot}5\mu$, the protoplasm is slightly granular, the nucleus centrally disposed, round and prominent, on account of its light colour. The structure of the nucleus is indiscernible. These bodies were nearly always encountered in groups of four in a row, as represented in Fig. 26, rarely in pairs or singly.

Once there occurred a pair of such bodies twice as large as the preceding, surrounded by a kind of capsule or membrane (Fig. 27).

Unfortunately I can say nothing regarding the nature of these bodies. Mr C. Dobell suggested they might be the spores of an Ascomycete.

REFERENCES.

BILLET, M. (1904). A propos de l'Hémogrégarine de l'émyde lépreuse (*Emys leprosa* Schw.) de l'Afrique du Nord. *C. R. Soc. Biol.* LVI.

CONOR, A (1912). Sur une Hémogrégarine karyolysante de *Naia hajae*. *Ibid.* LXXII.

DOBELL, C. C. (1908). Some notes on the haemogregarines parasitic in snakes. *Parasitology*, I. 4.

—— (1910). On some parasitic Protozoa from Ceylon. *Spol. Zeylan.* VII, Pt XXVI.

DOFLEIN, F. (1916). *Lehrbuch der Protozoenkunde.* 4 Aufl. Jena.

DUTTON, J. E., TODD, J. L. and TOBEY, E. N. (1907). Concerning certain parasitic Protozoa observed in Africa. *Ann. Trop. Med and Parasitol.* I.

FLU, P. (1910). Über Hämogregarinen im Blute Surinamischer Schlangen. *Arch. f. Protistenk.* XVIII.

LANGMANN, G. (1899). On haemosporidia in American reptiles and batrachians. *New York Med. Journ.* Jan. 7.

LAVERAN, A. et PETTIT, A. (1911). Sur une Hémogrégarine de la vipère à cornes. *C. R. Soc. Biol.* LXX.

LUTZ, A. (1901). Ueber die Drepanidien der Schlangen. Ein Beitrag zur Kenntniss der Hämosporidien. *Centralbl. f. Bakter. etc.* XXIX, 1 Abth.

MACFIE, J. W. S. (1914). Notes on some blood parasites collected in Nigeria. *Ann. Trop. Med. and Parasitol.* VIII.

MINCHIN, E. A. (1907). On a haemogregarine from the blood of a Himalayan lizard (*Agama tuberculata*). *Proc. Zool. Soc. London.*

—— (1910). Report on a collection of blood parasites made by the Sleeping Sickness Commission, 1908–9, in Uganda. *Rep. Sleep. Sickness Commission of the R. S.* London.

NERESHEIMER, E. (1909). Über das Eindringen von *Lankesterella* spec. in die Froschblutkörperchen. *Arch. f. Protistenk.* XVI.

NÖLLER, W. (1912). Über eine neue Schizogonie von *Lankesterella minima* Chaussat (= *L. ranarum* Lank.). *Arch. f. Protistenk.* XXIV.

PLIMMER, H. G. (1912). On the blood parasites found in animals in the Zoological Gardens during the four years 1908–1911. *Proc. Zool. Soc.* London.

PROWÀZEK, S. v. (1907). Untersuchungen über Hämogregarinen. *Arb. a. d. Kaiserl. Gesundheitsamte*, XXVI, H. 1.

REICHENOW, E. (1910). *Haemogregarina stepanowi*. Die Entwicklungsgeschichte einer Hämogregarine. *Arch. f. Protistenk.* XX.

—— (1912). Die Hämogregarinen. In *Handbuch der Pathogenen Protozoen* (hrsg. von Prowazek). 5 Lief. Leipzig.

ROBERTSON, M. (1908). A preliminary note on Haematozoa from some Ceylon reptiles. *Spol. Zeylan.* V, Pt XX.

—— (1910). Studies on Ceylon Haematozoa. No. II. Notes on the life cycle of *Haemogregarina nicoriae* Cast. and Willey. *Quart. Journ. Micr. Sci.* LV, Pt 4.

SAMBON, L. W. (1908, 1909). The Haemogregarines of Snakes. *Journ. Trop. Med. and Hyg.* XI and XII.

SCHUBOTZ, H. (1913). Untersuchungen an parasitischen Protozoen aus Äquatorial-Afrika. I Teil: Hämogregarinen. *Ergebn. 2te Deutsch. Zentral-Afrika-Exped.* 1910–1911, I, 1.

SEIDELIN, H. (1911). Notes on some blood parasites in reptiles. *Ann. Trop. Med. and Parasitol.* V.

SERGENT, E. (1918). Une hémogrégarine de *Vipera libertina* L. d'Algérie. Début de l'évolution de cette Hémogregarine chez un Acarien. *Bull. Soc. Path. Exot.* XI.

SHORTT, H. E. (1917). Notes on two haemogregarines of cold-blooded vertebrates. *Ind. Journ. Med. Res.* IV. 3.

SIMOND, P. (1901). Contribution a l'étude des Hématozoaires endoglobulaires des reptiles. *Ann. Inst. Pasteur*, XV.

STEBBINS, J. (1905). On the occurrence of a large sized parasite of the Karyolysus order in the blood of *Rana clamata*. *Centralbl. f. Bakter. etc.* XXXVIII, 1 Abth.

STEVENSON, A. C. (1911). A few notes on the Protozoa parasitic in *Bufo regularis* in Khartoum. *Wellcome Res. Lab.*, Rep. 4, v. A.

WENYON, C. M. (1908). Report of travelling Pathologist and Protozoologist. *Ibid.* Rep. 3.

WOODCOCK, H. M. (1912). Notes on Sporozoa. Nos. II, III, and IV. *Quart. Journ. Micr. Sci.* LVIII, Pt 1.

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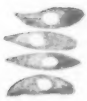
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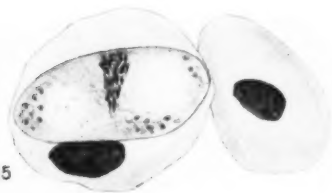
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EXPLANATION OF PLATE XVIII.

All the preparations were drawn by means of Leitz's camera lucida, at a magnification of about 1500 diameters (Leitz, oc. 4, Hom. imm. $\frac{1}{12}''$).

Figs. 1 to 3: from *Bitis gabonica*.

Fig. 1. Small (young) form of haemogregarine.

Figs. 2, 3. Trophozoites.

Figs. 4 to 7: from a *tree snake* (No. 5).

Fig. 4. Normal erythrocyte.

Figs. 5, 6, 7. Haemogregarines. In Fig. 5 the parasite is closely adjacent to the vacuolized nucleus of the blood corpuscle. In Fig. 7 the nucleus of the latter is hypertrophied.

Figs. 8 to 18: from *Bufo No.* 9.

Fig. 8. Normal erythrocyte.

Figs. 9, 10, 11. Merozoites: free, entering and leaving the erythrocytes.

Figs. 12, 13. Trophozoites.

Fig. 14. Trophozoite, one limb of which is being reduced. Granules of volutin visible.

Figs. 15, 16. Full grown trophozoite (schizont). Volutin visible. Dark hood at one of the poles visible. In Fig. 16 the nucleus of the erythrocyte has broken up into two parts.

Figs. 17, 18. Stages of maturation of the schizont. In Fig. 17 the parasite is seen to be leaving the corpuscle in which the abandoned capsule is visible.

Figs. 19 to 25: from *Bufo No.* 6.

Fig. 19. Merozoite.

Figs. 20, 21. Same: growth and bending.

Fig. 22. Mature form (trophozoite).

Fig. 23. Same: fusion of the two limbs.

Figs. 24, 25. Schizonts.

Figs. 26, 27. Bodies of undetermined origin.

FISH MYXOSPORIDIA FROM PLYMOUTH.

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(With 6 Text-figs.)

During the summer of 1914 and during the months January to April, 1920, I have been able to investigate the Myxosporidian parasites of fish occurring at Plymouth, and I am glad to take this opportunity of thanking the Ray Lankester Trustees for their grant in aid of this research, and also to express my gratitude for the very valuable and kind assistance afforded me by the Director, Dr E. J. Allen, and staff of the Marine Biological Station at Plymouth, where the researches were carried out. The assistant Mr Smith helped me very considerably in the identification of the fish examined.

I have been unable as yet to work out the material fully, especially the interesting developmental stages, and as it may be some time before I am able to do this, it may be useful to give a list of the forms found in fish met with at Plymouth, and to describe briefly some new forms.

Quite a number of new hosts were found and it is a matter of difficulty at present to decide whether a parasite found in a new host is a new species or not. The same difficulty occurs of course in other groups of parasites, notably Trematoda and Anoplura, and while some species are certainly found in many different hosts, others apparently are very restricted in their choice of hosts, even when abundant opportunities occur for infection. In this paper a very conservative attitude has been adopted with regard to possible new species. When a form has been found with spores which agreed with a published description, the parasite has been given the name of the earlier described species, although found in a different host. The plasmodial stages do not show sufficiently clear distinctions to serve for specific or even generic characteristics, except in rare cases like the pigmented plasmodium of *Chloromyxum*.

Some of the forms described under pre-existing names may be found subsequently to be distinct species, but with our present defective knowledge of life histories in this group it is better to describe the same species as occurring in different hosts rather than create new specific names which might have to be abandoned later. Some species like *Chloromyxum leydigi* have been recorded already from many different hosts, and it will be seen that a form indistinguishable from *Myxidium incurvatum* is here recorded from several different hosts. On the other hand, there appear to be some species which

are more restricted in occurrence. One case is that of *Sphaeromyxa ovata* which occurred in all of the three specimens of *Onos tricirratus* examined, but in only one of 46 specimens of *Onos mustela* which came from the same locality. In the one case in which it did occur, it was present in very small numbers only, as though it had not been able to establish a strong infection. It has been held that these spores are abnormal spores of *Sphaeromyxa balbianii*, but the spores are very dissimilar and were not found together by me. Another example of restriction in distribution is shown by *Myxidium incurvatum*, which was found in five out of 19 specimens of *Blennius pholis*, but not in one of 30 specimens of *Gobius* (various species) taken from the same rock pools. Yet *Myxidium incurvatum* is found in a large number of different hosts, and the Gobies must sometimes take up spores of this and other species. It can be suggested at least that not all species of fish are equally susceptible, and it appears likely that some species of Myxosporidia are specific to certain hosts, but their mere occurrence in different hosts cannot be taken as proof of specific difference, without some accompanying difference in form, size of spore, or other characteristic feature. Therefore it will be found that in this paper specimens have been identified as far as possible with pre-existing species, and also as far as possible with the species mentioned by Labbé in *Das Tierreich*. It would be a convenience to parasitologists if in the case of parasites a trinomial system of nomenclature for animals and plants could be used, indicating the specific character of the parasite and also the host from which it was obtained.

In the following list the fish hosts have been named for the sake of uniformity according to Dr Smitt's edition of Fries, Ekström and Sundevall's *Scandinavian Fishes*.

Host	Examined	Negative	Infected	Parasite
Agonus cataphractus ...	2	2	0	
Anguilla vulgaris	1	1	0	
Blennius gattorugine ...	5	5	0	
Blennius ocellaris	2	1	1	*Myxidium incurvatum* Thél. + *Ceratomyxa arcuata* Thél.
Blennius pholis	19	14	5	*Myxidium incurvatum* Thél.
Bothus maximus	14	13	1	*Myxidium incurvatum* Thél.
Callionymus lyra	15	0	15	*Myxidium incurvatum* Thél. (13). *M. incurvatum* + *Ceratomyxa arcuata* Thél. (2).
Capros sanglier	1	0	1	*Ceratomyxa lata* sp. n.
Clupea pilchardus	17	6	11	Plasmodium only (1). *Ceratomyxa truncata* Thél. + *Coccomyxa morovi* Léger and Hesse.
Cottus bubalis	3	0	3	*Ceratomyxa dubia* sp. n. (3). *Plistophora typicalis* Gurley in liver (1). *Chloromyxum quadratum* Thél. in muscles (1).
Gadus luscus	6	6	0	

Host	Examined	Negative	Infected	Parasite
Gadus merlangus	8	4	4	*Myxidium sphaericum* Thél. (3). *Ceratomyxa arcuata* Thél. (1).
Gadus minutus	5	4	1	*Sphaeromyxa longa* sp. n. + *Myxidium sphaericum* Thél.
Gadus pollachius	13	13	0	
Gastraea spinachia ...	1	1	0	
Gobius flavescens	4	4	0	
Gobius minutus	11	11	0	
Gobius paganellus	15	15	0	
Labrus (Crenilabrus) melops	4	4	0	
Labrus mixtus	1	1	0	
Lepidorhombus whiff (megastoma)	2	1	1	Plasmodia only.
Lophius piscatorius ...	4	2	2	*Ceratomyxa appendiculata* Thél. (?)(2). All four with *Glugea lophii* on nerves.
Merlucius merluccius ...	1	1	0	
Molua molva	1	0	1	*Ceratomyxa* sp.? No free spores seen.
Mustelus vulgaris	5	4	1	*Chloromyxum leydigi* Ming. Plasmodia only.
Nerophis lumbriciformis ...	9	9	0	
Onos mustela	46	44	2	*Sphaeromyxa balbianii* Thél. (1). *Sphaeromyxa ovata* sp. n. rare spores (1).
Onos tricirratus	3	0	3	*Sphaeromyxa ovata* sp. n.
Pholis gunnellus	1	1	0	
Platophrys laterna ...	2	1	1	*Myxidium incurvatum* Thél. + *Ceratomyxa arcuata* Thél.?
Pleuronectes flesus ...	8	7	1	*Myxidium intermedium* sp. n.
Pleuronectes limanda ...	15	10	5	*Ceratomyxa sphaerulosa* Thél.
Pleuronectes microcephalus	3	0	3	*Ceratomyxa lata* sp. n.
Pleuronectes platessa ...	8	8	0	
Ramphistoma belone ...	1	1	0	
Rhina squatina	3	3	0	
Roccus labrax	1	0	1	*Ceratomyxa arcuata* Thél.
Scomber scombrus ...	15	3	12	Plasmodia only.
Scylliorhinus canicula ...	5	5	0	
Scylliorhinus stellaris ...	5	3	2	*Chloromyxum leydigi* Ming.
Solea variegata	3	2	1	Plasmodia only. *Ceratomyxa* sp.?
Solea vulgaris	2	2	0	
Squalus acanthias ...	5	0	5	*Chloromyxum leydigi* Ming.
Syngnathus typhle... ...	5	5	0	
Trigla gurnardus	13	13	0	
Zeus faber	7	7	0	

DESCRIPTION OF SPECIES.

Ceratomyxa lata sp. n. (Fig. 1). Host: *Capros sanglier*. Habitat: Gall bladder. Spore: $19\mu \times 7\mu$, crescentic in shape, ends rounded, polar capsules large, not marginal. The proportions and shape of this form distinguish it from any other, the nearest to it being *C. coris* Georg. (from *Coris julis*), which is less crescentic in form and from a different host and locality. A similar form was found in *Pleuronectes microcephalus*, and is given provisionally the same name.

Ceratomyxa dubia sp. n. (Fig. 2). Host: *Cottus bubalis*. Habitat: Gall bladder. Spore: 17·5μ × 8μ. Polar threads 30μ long. This form approaches *Leptotheca* in proportions, but sporoplasm does not fill spore, and the organism is therefore named as a Ceratomyxa, but is, like *C. coris* Georg. and to a less extent *C. lata*, an intermediate form between these two closely related genera.

Myxidium intermedium sp. n. (Fig. 3). Host: *Pleuronectes flesus*. Habitat: Gall bladder. Spore: 12μ × 6–7μ, broad ~-shaped like *M. incurvatum* Thél.,

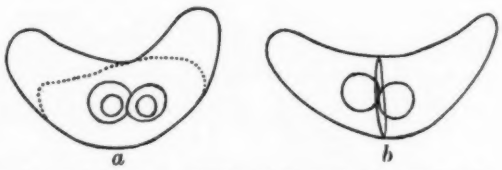

Fig. 1. *Ceratomyxa lata* sp. n. ×1900. (*a*) Spore showing extent of sporoplasm. (*b*) Spore showing sutural line.

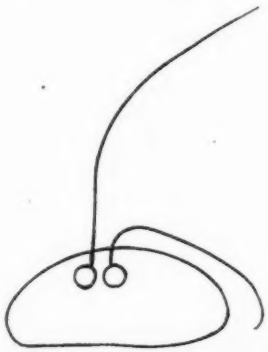

Fig. 2. *Ceratomyxa dubia* sp. n. ×1900. Polar filaments extruded.

Fig. 3. *Myxidium intermedium* sp. n. ×1500.

but larger and from a different host, no Myxidium having been recorded from *Pleuronectes*. Size alone is not a reliable guide to species as there is apparently great variation amongst specimens from different localities, though not much in any one infection. Auerbach gives the size of *Myxidium bergense* spore as 16·2–19μ long × 7–9μ wide, but specimens from *Gadus virens* caught at Millport on the Clyde, which I have carefully drawn with camera lucida and compared with a Zeiss 1/100 mm. scale drawn under the same conditions, are all very near to the measurements 12·5μ × 5μ.

Sphaeromyxa longa sp. n. (Fig. 4). Host: *Gadus minutus*. Habitat: Gall bladder. Spore: $20\mu \times 5\mu$, similar in form to that of *S. balbianii* Thél., but much longer and from a different host. This form was compared with *S. balbianii* which was obtained from *Onos mustela* and the *S. balbianii* spores were consistently smaller, $16\mu \times 5\mu$. There was very little variation in the size of spores in either case when carefully measured, not more than $1{\cdot}5\mu$ in length. *S. longa* was found in association with *Myxidium sphaericum* Thél., the spores of *S. longa* being more numerous than those of *M. sphaericum*.

Sphaeromyxa ovata sp. n. (Fig. 5). Host: *Onos tricirratus*. Habitat: Gall bladder. Spore: $13\mu \times 6{\cdot}5\mu$, oval with round ends, some slightly curved in one plane, polar capsules large, terminal. This form resembles *Cystodiscus*

Fig. 4. *Sphaeromyxa longa* sp. n. ×1500.

Fig. 5. *Sphaeromyxa ovata* sp. n. ×1900.

Fig. 6. *Chloromyxum quadratum*. (*a*) Spore from above. (*b*) Spore with polar filaments extruded. ×1900.

immersus Lutz parasitic in Amphibia, but *C. immersus* is said to be 9–10μ wide. Occasionally an abnormal spore of *S. ovata* can be found measuring $10\mu \times 8\mu$, which is nearer the proportions of *C. immersus*, but is smaller. Georgevitch describes this form as a polymorph of *S. balbianii*, but no spores of *S. balbianii* were found in the three specimens of *Onos tricirratus* containing *S. ovata*. One out of 46 specimens of *Onos mustelus* showed a slight infection by this parasite, spores being very rare. This specimen was not infected with *S. balbianii*.

Chloromyxum quadratum Thél. A figure of *Chloromyxum quadratum* from the muscles of *Cottus bubalis* is subjoined to show the extruded polar filaments and the four polar nuclei (Fig. 6).

REFERENCES.

AUERBACH (1910). *Die Cnidosporidien.* Leipzig.
—— (1912). *Zool. Anz.* XL.
AWERINZEW (1909). *Arch. f. Protistenk.* XIV.
DOFLEIN (1916). *Lehrbuch der Protozoenkunde.* 4th ed.
DUNKERLY (1915). *Proc. R. Phys. Soc. Edin.*
FRIES, EKSTRÖM and SUNDEVALL (1893–4). *History of Scandinavian Fishes.* 2nd ed. By F. A. SMITT.
GEORGEVITCH (1916). *Bull. Inst. Ocean. Monaco,* No. 322.
—— (1917). *Arch. Zool. Exp.* LVI.
JAMESON (1913). *Bull. Inst. Ocean. Monaco,* No. 273.
LABBÉ (1899). "Sporozoa" in *Das Tierreich.* Berlin.
LÉGER and HESSE (1907). *C. R. Acad. Sci.* CXLV.
PARISI (1913). *Atti della Soc. Ital. Sci. Nat.* LI.

THE CAPITULUM OF *PSOROPTES* (ACARINA)[1].

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(*From the Quick Laboratory, University of Cambridge.*)

(With 2 Text-figures.)

This short account of the capitulum and mouth-parts of *Psoroptes* has been prepared as a preliminary to investigations which are being made upon the bionomics of the Itch Mites. None of the published figures or descriptions are complete. The best is that of Mégnin.

The general appearance of the capitulum can be seen from Fig. 1, A and B. Viewed as a whole it is roughly pear-shaped and is inserted into the front part of the body. The dorsal surface (Fig. 1 B) is overhung by a fold of the general integument (*d.f.*, the dorsal fold or epistome), the ventral surface is free, except where it joins the body proximally. The basis capituli (*b.c.*) bears two pairs of setae on its ventral surface, a short pair arising near the palpal articulation[2] (*s* 1) and a longer pair situated closer to the mid-ventral line. The base of the palp is so shaped ventrally that it very nearly surrounds the origin of *S* 1. Both pairs of setae are directed downwards and forwards. Between the bases of the second pair (*s* 2) the surface of the basis capituli bears a minute keel-like structure (*k*) which protrudes strongly in front. The distribution of the sculpturing on the ventral surface of the basis capituli is shown in Fig. 1 A. There is a roughly rectangular bare area (*b.a.*) near the insertion of the basis capituli into the body, and the sculpturing does not extend quite as far forward as the point of origin of the second seta; there is no sculpturing on the dorsal surface of the basis capituli, nor does it extend far up the side.

The mouth-parts themselves are shown in Fig. 2. I can find no difference between those of the male and of the female. The chelicerae (Fig. 2 C) are long and finely chelate. Each of the limbs which form the chela is armed with two recurved hooks (*h*), curved ventrally. The base of the chelicera is wider and more thickly chitinized than the extremity, and extends back inside the capitulum to a point slightly behind the origin of seta 2. In the dead mite

[1] Work carried out with the aid of a grant from the Ministry of Agriculture and Fisheries.

[2] I refer to the second appendage of *Psoroptes* as palp in accordance with the usage of most of those who have studied the Ticks and Mites. On strict morphological grounds it should be called a "pedipalp," the name which is used for the second appendage of Arachnoidea in general.

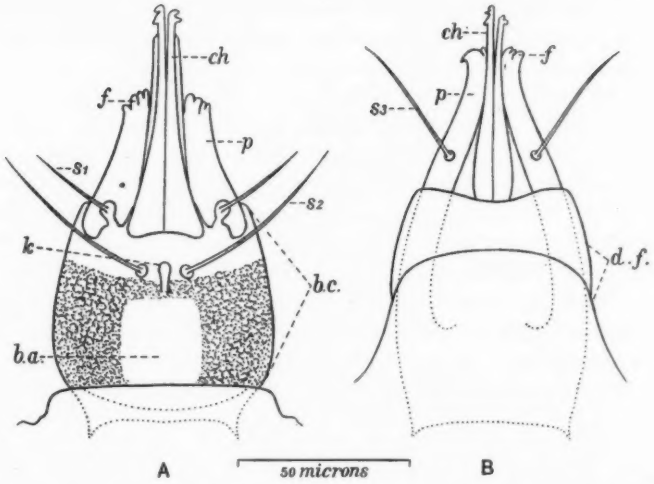

Figure 1. Capitulum of *Psoroptes equi*. A ventral and B dorsal aspects. *b.a.* bare area of basis capituli. *b.c.* basis capituli. *ch.* chelicera. *d.f.* dorsal fold of integument. *f.* finger-like processes of palp. *k.* keel-like structure. *p.* palp. *s* 1—3 setae. Camera lucida drawing, magnification × 500.

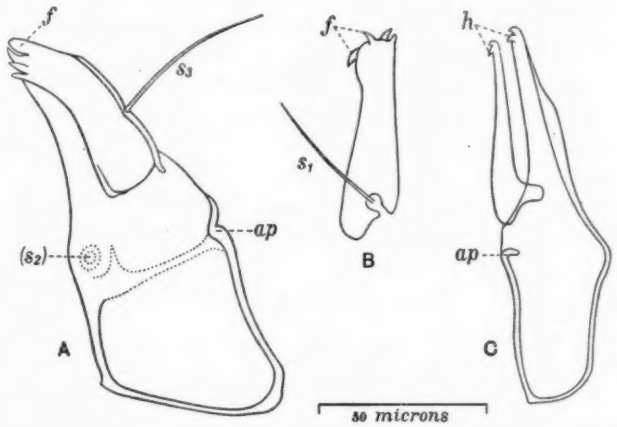

Figure 2. Mouth-parts of *Psoroptes equi*. A right palp, and basis capituli, median aspect after removal of chelicerae and left palp. B right palp, ventral aspect, with finger-like processes spread out. C chelicera seen laterally. *ap.* apodeme, for attachment of muscle. *f.* finger-like processes at end of palp. *h.* hooks on both members of chelicera. *s* 1 and 2 setae. (*s*2) base of second seta on ventrolateral aspect of basis capituli, seen by transparency. Camera lucida drawing, magnification × 500.

one of the chelicerae is nearly always more extended than the other, but they are equal in length. The palps (*p*) arise antero-laterally from the basis capituli, flanking the chelicerae. The base of each palp is excavated (Fig. 2 B) on the ventral surface so as almost to surround the origin of seta 1, which springs not from the palp but from the basis capituli. The dorsal surface of the palp is somewhat more heavily chitinized than the rest of the organ, and from it arises a short seta (*s*3) directed forwards and upwards. The extremity of the palp is armed with about five delicate finger-like processes (*f*), covered with extremely thin, soft chitin. In some dead specimens these are seen lying side by side (fig. 2 A), in others they are spread out from the ventral face of the extremity of the palp (fig. 2 B).

I have made no observations on the manner in which these organs are used by the living mite, and it is difficult to see how such observations could be made, for not only are the chelicerae extremely small and colourless, but also the mite carries its head tucked down between the bases of the first pair of legs. It may be assumed that the mite obtains a secure hold on the skin of the host by aid of its ambulacra and tarsal claws, and that it then steadies itself by applying the finger-like processes closely to the skin. It would then bring the chelicerae into action, probably using setae 1 and 2 as organs of touch.

Mr S. Hirst of the British Museum (Natural History) has been good enough to examine some of the material on which I have worked, and says that "they seem to be *Psoroptes equi* but are not very typical. It is probable that nearly all the forms of *Psoroptes* occurring on domestic animals are merely races or varieties of a single species."

REFERENCE.

MÉGNIN, P. (no date). *Les Acariens Parasites.* Paris: Masson & Cie.

ON A REMARKABLE NEW SPECIES OF *POROCEPHALUS* (*P. POMEROYI*, SP. N.) FROM THE FORE-GUT OF A NIGERIAN COBRA.

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(*From the Wellcome Bureau of Scientific Research, Endsleigh Gardens, London, N.W.* 1.)

(With 1 Text-figure.)

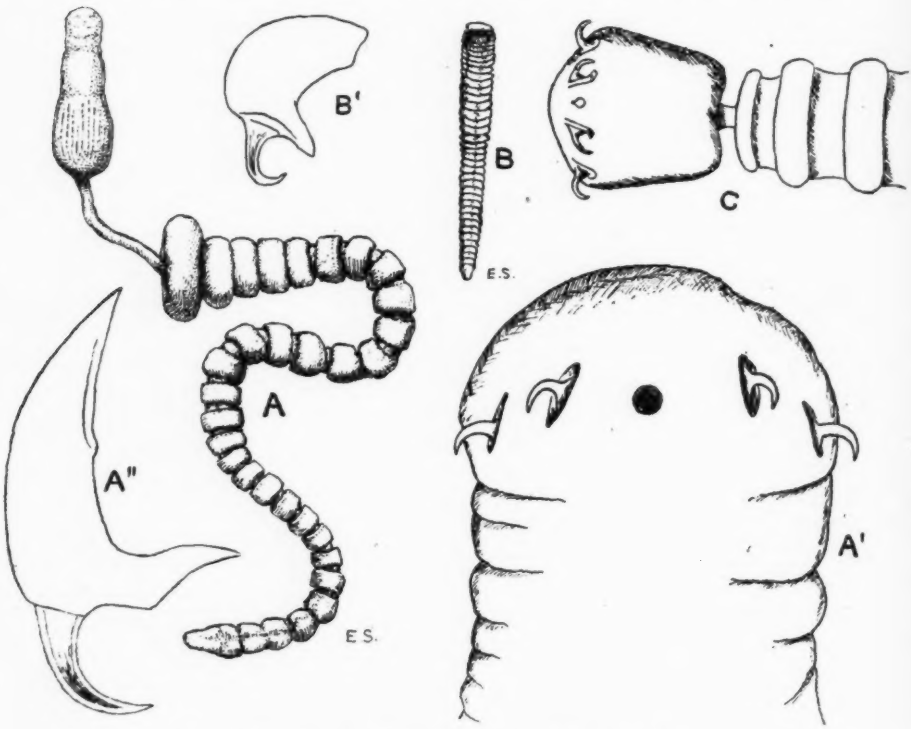
In August, 1920, Dr Andrew Balfour, C.B., C.M.G., Director of the Wellcome Bureau of Scientific Research, received from Mr A. Pomeroy, F.E.S., Official Entomologist in South Nigeria, a tube containing some parasites from the "fore-gut" of a Cobra (*Naia nigricollis* Reinh.) from Ilaro, South Nigeria. Dr Balfour kindly handed these specimens to me for examination. The specimens were few in number, comprising only ten Nematodes and the two examples of *Porocephalus* described in the present Note. One of these examples at once attracted my attention by reason of its remarkable external form (Text-figure 1, A). I have consulted most of the available literature dealing with *Porocephalus* and the figure which, so far as I have discovered, most nearly approaches that of the present specimen is that of *P. annulatus* Baird, supplied by Shipley (his Text-figure 5, p. 59) in his memoir on the Linguatulidae[1]. From my reproduction of Shipley's figure (Text-figure 1, C) it will be seen that *Porocephalus annulatus*, like the new species now to be described, has a very narrow "neck," but whereas in *P. annulatus* this neck is very short, in the new species it is comparatively very long; moreover, whereas in *P. annulatus* the cephalo-thorax (prosoma) is not longer than broad (or only slightly so in some specimens) and the first annulus of the "abdomen" (opisthosoma) is certainly no larger than succeeding annuli, in the new species the prosoma is roughly three times longer than it is broad and the first annulus is at least twice the size of the third at any succeeding annulus.

The External Features of a Female Specimen of Porocephalus pomeroyi, *sp. n.*

Text-figure 1 (A, A′, A″) illustrates clearly the principal external features of a female specimen of *Porocephalus pomeroyi*, sp. n. From these I have drawn up the following diagnosis: body white, cylindrical and divided distinctly into (1) a large prosoma, (2) a long narrow "neck" and (3) an annulated

[1] Shipley, A. E. (1898), *Archives de Parasitologie*. I, 52. Shipley provides a better figure in his article in the *Cambridge Natural History* Volume on Arachnida, p. 490, Fig. 256.

opisthosoma. In the type specimen the entire length of the body is 64 mm. The prosoma is elongated (8 mm. in the type-specimen), being one-eighth the length of the entire animal, and sac-shaped, with a maximum diameter (3 mm.) at least twice that of the opisthosoma posterior to the first two annuli. The "neck" is nearly equal in length (7 mm.) to the prosoma and in breadth



Text-figure 1. **A** Female specimen of *Porocephalus pomeroyi*, sp. n. (× 3.)
A' Anterior end of prosoma of the same specimen viewed ventrally. (× cir. 27.)
A" Hook of the same specimen. (× 80.)
B Small male specimen of Porocephalus (*pomeroyi?*). (× 3.)
B' Hook of the small male Porocephalus. (× 80.)
C Shipley's figure of *P. annulatus*. (× 4.) In Shipley's figure of this species in the *Cambridge Natural History* the prosoma is shown as being slightly more elongated.

I am indebted to Mr E. Schwarz Lenoir, artist to the Wellcome Bureau, for figures **A** and **B**.

(0·8 mm.) but little more than half the diameter of the hind portion of the opisthosoma. The opisthosoma in the type-specimen consists of 32 annuli, including the terminal or anal "segment." The first annulus is much the largest, exceeding in diameter the prosoma, and in length all the succeeding annuli. The second annulus is about two-thirds the diameter of the first

(slightly less than that of the prosoma) and is shorter. Succeeding annuli are smaller and very gradually diminish in size to the posterior extremity, the terminal annuli being about two-thirds the diameter of the anterior annuli. The anal "segment" is about twice as long as the penultimate annulus, though of the same diameter. The prosoma bears at its anterior end on the ventral surface the usual four chitinous hooks (curved, elongated, acute) and the small median sub-terminal mouth. Anus terminal. Habitat: "fore-gut" of Cobra (Naja sp.?), Ilaro, South Nigeria. The type-specimen is a female, preserved in the collection of the Wellcome Bureau of Scientific Research.

I may remark that I could not observe any "stigmata" (orifices of epidermal glands) on the surface of the skin, nor papillae; nor could I make out the position of the female genital aperture, though, judging from appearances when the specimen was cleared in creosote, it is probably situated ventrally near the anus. The drawing of the chitinous hook was made from the creosote-cleared specimen, but the precise outline of the basal fulcrum and process was not very easy to observe. The creosote also revealed hundreds of eggs contained in the uterus extending from the sixth annulus back to the anus. Since it is desired to keep intact this one specimen of *P. pomeroyi* (the only specimen at present known to exist), I am unable to describe the internal anatomy, but this I hope to do should additional specimens be forthcoming.

The External Features of a small Male Specimen of Porocephalus (pomeroyi?) *from the same Cobra.*

Text-figure 1 (B, B′) shows the more general external characters of this small specimen, obtained from the same Cobra, and in close juxtaposition to the female *Porocephalus pomeroyi* just described. The entire body measured 12 mm. in length and consisted of the small conical prosoma bearing the usual hooks and mouth, and 37 annuli (not clearly indicated in the figure), including the small pointed anal "segment." The body is slightly flattened in the region of the first ten annuli. The hooks are as shown in the figure. When cleared in creosote the specimen was seen to be a mature male. The male aperture was situated anteriorly and ventrally at the level of the third annulus.

In general characters and in size the specimen somewhat resembles the figure of *Porocephalus aonyces* Macalister, provided by Shipley (*loc. cit.* Text-figure 6), but is probably not identical with that species, which is parasitic in the peritoneal cavity of the large Indian Otter (*Aonyx cinerea*), is 17–20 mm. in length (sex supposed to be female) and has 30 annuli.

On the Possible Relationship between the two Specimens of Porocephalus *above described.*

Mr Pomeroy remarks in his letter concerning these two specimens of Porocephalus that "they seem to be in coitu"—a suggestion which I presume was based on the facts that they were of opposite sexes and that they were in close proximity in the "fore-gut" of the Cobra. Other evidence in favour

of this suggestion is to be found in their relative dimensions. The female specimen (64 mm.) was slightly more than five times as long as the male. This is also the case in other species of Porocephalus. Thus Spencer[1] states that in *P. teretiusculus* the female is nearly five times as long as the male; Miss Hett[2] similarly mentions that in Raillietiella a female measures 40 mm. and a male 10 mm.; Harley[3] says that the largest female specimen (found in the lungs of an Egyptian Cobra) of "Pentastoma annulatum" was $4\frac{3}{4}$ inches long and a male (found in the nasal fossa of the same Cobra) $1\frac{1}{8}$ inch long; in *P. lari*, Mégnin, the female measures 6 cms. and the male about 1 cm., and in most other species of Porocephalus the female is at least twice as long as the male.

Against this view that these two specimens are a female and male of the same species are the facts that they are of very different shape externally, that the female has 32 annuli and the male 37, and that the hooks are perhaps slightly different in form. However, the exact number of segments which compose the opisthosoma cannot be regarded as a safe character on which to found specific distinctions, nor are minute differences in shape of the hooks of more value, and I think it is highly probable that the two specimens of Porocephalus above described are the female and male forms of the same species. If this be so, then these two specimens present the most marked form of sexual dimorphism yet discovered in the Linguatulidae.

Note on the Habitat of Porocephalus pomeroyi.

By "fore-gut" I assume Mr Pomeroy to mean the anterior part of the intestine, and not the mouth-cavity, throat or oesophagus of the Cobra. This assumption is borne out by the fact that with the two Porocephalus individuals ten Nematodes (the smallest 12 mm. long) were found. I need hardly point out that this situation, if correctly stated[4], is an unusual one, the vast majority of Linguatulids being found either in the frontal sinuses, nasal cavity, trachea, lungs or body-cavity or in the substance of certain organs (muscles, liver, kidney, spleen) of Vertebrates other than fishes.

Postscript. (8. x. 1920): Mr Pomeroy informs me that the two specimens of *Porocephalus* above described "appeared to be joined together by a ligament and were not separated when I removed them from the snake." Mr Pomeroy encloses a rough sketch from memory of the way in which the two specimens were united, from which it would appear that a filamentous connection extended between the posterior sexual aperture of the large female and the exterior sexual aperture of the small male; this connection seems to prove that the pair belong to the same species.

Mr Pomeroy also confirms his previous statement that the pair were found in the intestine "about the middle of the snake, and not as I have found them before in the first part of the fore-gut."

[1] Baldwin Spencer (1893), *Quart. Journ. Micr. Science*, XXXIV, 1.

[2] Hett, M. L. (1915), *Ibid.* LXI, 185.

[3] Harley, G. (1857), *Proc. Zool. Soc. London*, Part 25, p. 115.

[4] Wyman (*Bost. Soc. Nat. Hist.*, Sept. 17, 1845) mentions *Porocephalus armillatus* as occurring in the intestines of a Python.

ON SOME FILARIID PARASITES OF CATTLE AND OTHER RUMINANTS[1].

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(With 7 Text-figures.)

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INTRODUCTION.

This memoir presents the results of the study of a number of specimens of Filariid worms from Cattle and other Ruminants which had accumulated during the last few years from various collections of Nematodes, sent to me for identification and laid aside for further investigation.

I am indebted to Professor G. H. F. Nuttall, F.R.S., for two of these collections: (1) Helminths from Wassein, Burma, sent by Dr H. H. Marshall, and (2) from British East Africa, collected by Mr R. E. Montgomery, of the Nairobi Veterinary Pathological Laboratory. A third collection was obtained from Northern Rhodesia by Mr H. E. Hornby, Government Veterinary Surgeon, whilst my material also includes some specimens sent by the Department of Agriculture, Reduit, Mauritius.

The Filariids from these various sources all proved to belong to the group of species now separated from the genus *Filaria s. str.* and placed by Railliet and Henry (1911) in the genus *Setaria* Viborg 1795. Altogether thirteen species have so far been referred to this genus, of which ten have been recorded as parasites of Ruminants; of the latter only two are at all well known, namely *Setaria labiato-papillosa* (Aless.) and *S. equina* (Abildg.), the second species essentially a parasite of Equines but stated to have been observed in Cattle also (Stossich, 1897). The remaining forms from Ruminants have for

[1] In the absence of the Author the proof sheets of this paper were passed by the Editor.—G.H.F.N.

the most part been recorded on single occasions only and the majority are very incompletely described, so much so as to be practically unrecognisable.

The material at my disposal was found to consist of four species, two of these are described as new, the others referred to the species *S. labiato-papillosa* (Aless.) and *S. digitata* (v. Linst.). Whilst describing the two new forms I have considered it advisable to add a brief account of the latter species also, since even the commoner parasite of Domestic Cattle is very shortly described in the text-books and very few records of the measurements of the various organs seem available. I have also added a short diagnosis of the genus *Setaria*.

Genus SETARIA Viborg 1795 (non Oken 1815).

Hamularia Treutler 1795, Stiles 1907.

Filaria auct. p.p.

GENERIC DIAGNOSIS. *Filariidae:* Body cylindrical, filiform, considerably attenuated at the posterior extremity in both sexes. Cuticle finely striated transversely. Mouth surrounded by a chitinous peribuccal ring, notched laterally and usually also dorso-ventrally so as to give the impression of two or four projecting teeth. Four submedian head-papillae always present, a pair of lateral papillae probably always occur as well but are frequently difficult to see. Oesophagus consisting of two parts: a short, narrow anterior ("vestibule" of some authors) and a much longer and thicker posterior region.

Male smaller than the female, its attenuated caudal extremity ending in a close spiral. Preanal and postanal papillae present as well as in the majority of species a pair of small lateral appendices close to the posterior extremity. Spicules very unequal, the longer consisting of two parts the posterior of which is largely membranous, the shorter curved.

Female with caudal region coiled in a loose spiral and bearing a pair of lateral appendices close to the extremity. Vulva near the anterior end of the body. Eggs thin-shelled. Ovoviviparous.

Parasitic in the peritoneal cavities of Mammals.

Type Species: *Setaria equina* (Abildgaard 1789), Railliet and Henry, 1911.

Setaria labiato-papillosa (Aless. 1838).

This common parasite of Ruminants is represented in my material by specimens from Domestic Cattle and the Bush-Buck in British East Africa and from the Stag (*Cervus hippelaphus*) in Mauritius.

SPECIFIC DIAGNOSIS. *Setaria:* Body tapering gradually to the anterior extremity. Head rounded, not separated from the remainder of the body.

Peribuccal ring prominent, much elongated dorso-ventrally and deeply notched laterally in such a way as to give the appearance in a side view (Text-fig. 1A) of strong dorsal and ventral teeth separated by a wide depression from the centre of which arises a semi-circular lip-like elevation. Each tooth is in its turn indented so as to form two cusps, visible when the head is

viewed from the dorsal or ventral surface (Text-fig. 1B), they are usually better developed in the females than in the males.

Submedian head-papillae small, lateral papillae very inconspicuous.

The anterior region of the oesophagus measures 0·6–0·85 mm. in length, whilst the posterior region has a length of 6·5–7·3 mm.

Male: 40–46 mm. in length with a maximum thickness of about 0·6 mm.

The tail region is coiled in a close spiral, the cloaca situated 0·19–0·23 mm. from the posterior extremity. The latter is provided with a rounded terminal

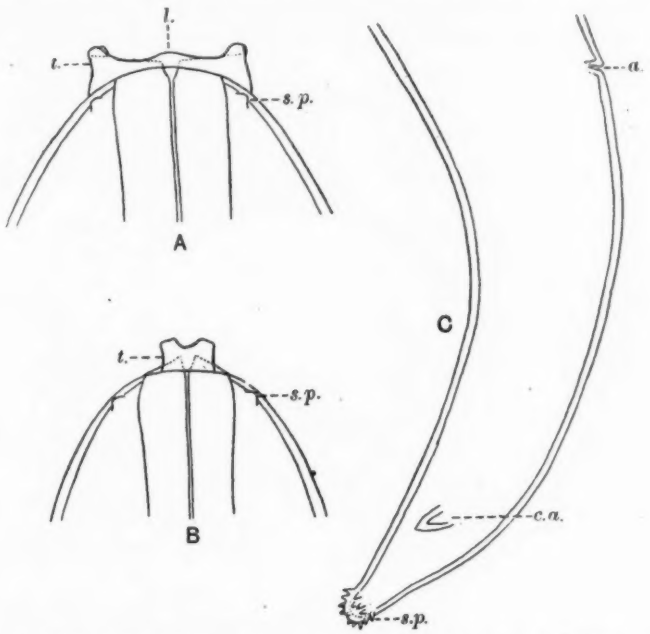

Fig. 1. *Setaria labiato-papillosa* (Aless.). A. Head in lateral view. B. Head in dorsal view. C. Lateral view of caudal extremity of female. ×220.

knob close in front of which are situated a pair of conspicuous lateral appendices (Text-fig. 2). There are four pairs of preanal and four pairs of postanal papillae.

The spicules are very unequal, the larger measuring 0·33–0·37 mm. in length and consisting of a long cylindrical anterior region followed by a much shorter terminal region which is largely membranous. The shorter spicule (Text-fig. 2) is stout, slightly curved and terminates in a sharp point.

Female: 62–94 mm. in length, with a maximum breadth of 0·7–0·8 mm.

The caudal region is coiled in a loose spiral and terminates in a knob-like extremity surrounded by an irregular ring of pointed spines, the latter very

variable in number (Text-fig. 1 C). The lateral appendices of the tail are large and situated about 0·12 mm. from the posterior extremity.

Anus about 0·5 mm. from the posterior end of the body.

Vulva somewhat variable in position, 0·45–0·75 mm. from the anterior extremity.

Eggs thin-shelled, measuring 0·03–0·046 × 0·02–0·03 mm.

Setaria digitata (v. Linstow 1906).

v. Linstow's type-specimens were obtained from *Bos indicus* in Ceylon; I have referred to this species a number of worms collected by Dr Marshall

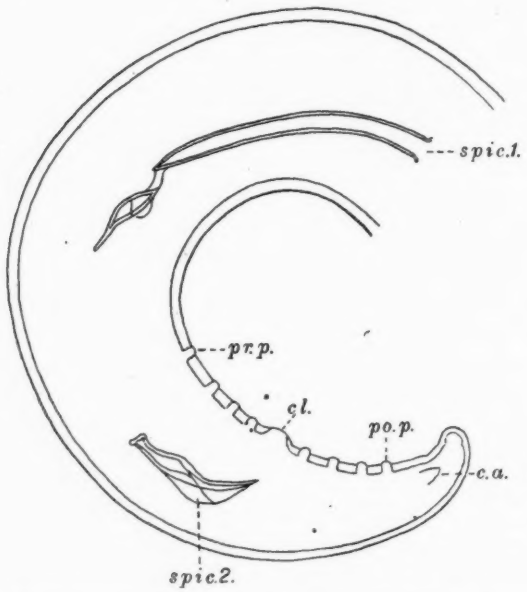

Fig. 2. *Setaria labiato-papillosa* (Aless.). Lateral view of caudal extremity of male. ×220.

from the peritoneal cavity of Domestic Cattle at Wassein, Burma. Unfortunately only female specimens are represented in the material; these agree fairly well in their measurements with those described by v. Linstow. I have however given a somewhat different interpretation to the structure of the organs surrounding the mouth.

The species is evidently very closely allied to *S. equina* (Abildg.), the type species of the genus.

Specific diagnosis. *Setaria:* Head not separated from the body which tapers gradually to the anterior extremity.

Peribuccal ring resembling that of the preceding species, the lateral notches are however less deep and the lip-like chitinous projections from their centres are better developed and frequently almost triangular in shape (Text-fig. 3 A), in which case they have the appearance of lateral teeth. The dorsal and ventral teeth are notched so as to present two cusps (Text-fig. 3 B).

Sub-median papillae small and rounded, lateral papillae were not seen.

Female: 62–78 mm. in length, maximum breadth of the body 0·5–0·68 mm.

The anterior region of the oesophagus measures 0·53–0·8 mm. in length, the posterior region 6·5–8 mm.

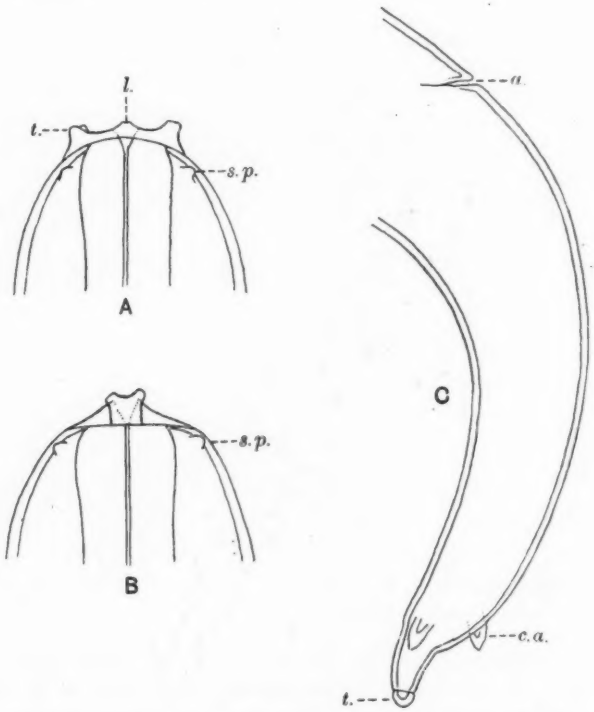

Fig. 3. *Setaria digitata* (v. Linst.). A. Head in lateral view. B. Head in dorsal view. C. Lateral view of caudal extremity of female. × 220.

The posterior extremity of the loose caudal spiral terminates in a smooth knob, a pair of large lateral appendices are situated 0·1 mm. from the end of the body (Text-fig. 3 C).

Anus 0·4–0·48 mm. from the tip of the tail.

Vulva 0·5–0·65 mm. from the anterior extremity. Eggs thin-shelled, 0·032–0·045 mm. × 0·018–0·02 mm.

Setaria Marshalli sp. n.

Among the specimens of *S. digitata* collected by Dr Marshall from Cattle at Wassein, Burma, was a single female *Setaria* which differs markedly from all known forms of the genus and which I take as the type of a new species, named after the collector. The specimen was unfortunately not in a good state of preservation and the description is therefore incomplete in a few particulars.

SPECIFIC DIAGNOSIS. *Setaria:* Head not separated from the rest of the body and appearing truncated anteriorly.

The peribuccal ring is elongated in the dorso-ventral direction and is very deeply notched laterally. The lip-like lateral projections described in *S. digitata*

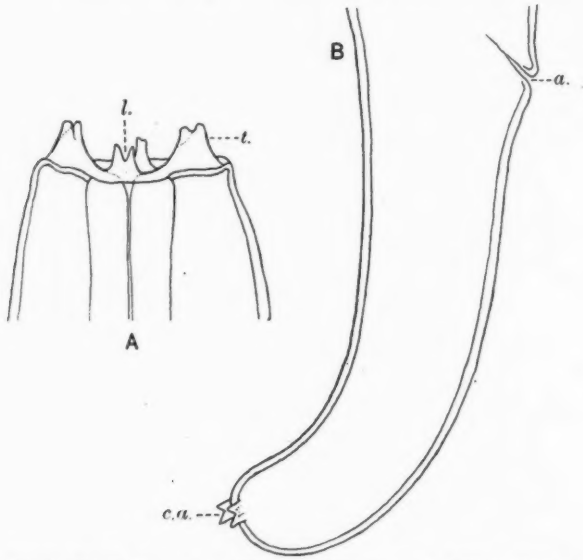

Fig. 4. *Setaria Marshalli* sp. n. A. Head in lateral view. B. Lateral view of caudal extremity of female. ×220.

and *S. labiato-papillosa* are in this species exceptionally prominent and markedly tooth-like, being themselves indented at their apices (Text-fig. 4 A); they bear a strong resemblance to the dorsal and ventral teeth which are strong and provided with two cusps.

Head papillae were not observed.

Female: 90 mm. in length, with a maximum breadth of about 0·6 mm.

The oesophagus has a total length of 9 mm.

Anus 0·45 mm. from the posterior extremity, the latter is rounded and slightly curved in the dorsal direction (Text-fig. 4 B). The lateral appendices are bifurcated and situated very close to the posterior extremity of the body.

Vulva about 0·6 mm. from the anterior extremity.

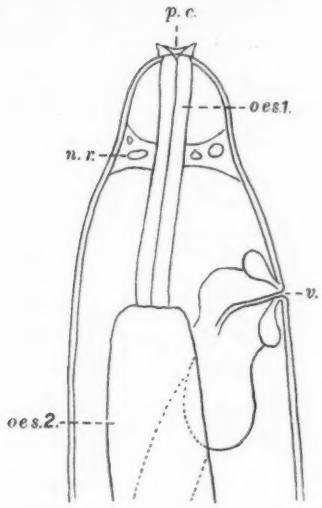

Fig. 5. *Setaria Hornbyi* sp. n. Anterior extremity of female. ×55.

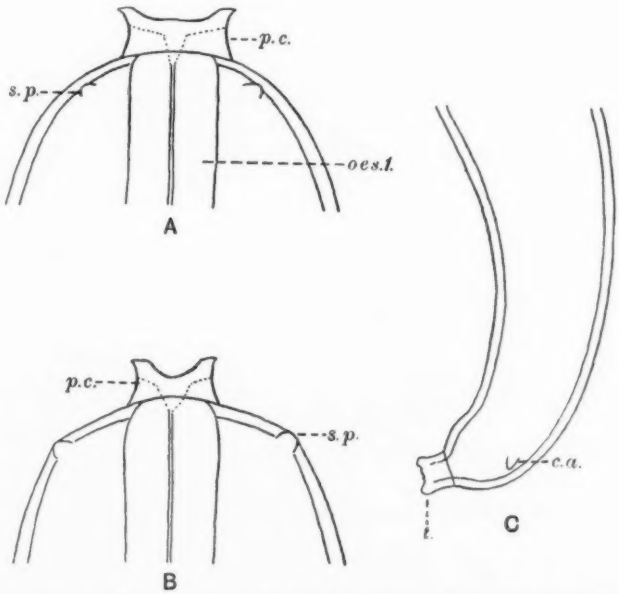

Fig. 6. *Setaria Hornbyi* sp. n. A. Head in lateral view. B. Head in ventral view. C. Caudal extremity of female. ×220.

Setaria Hornbyi sp. n.

Male and female specimens of a large species of *Setaria* were obtained by Mr H. E. Hornby from the Sable Antelope, *Hippotragus niger*, in Northern Rhodesia. The worms were found on the surface of the liver, in the mediastinal

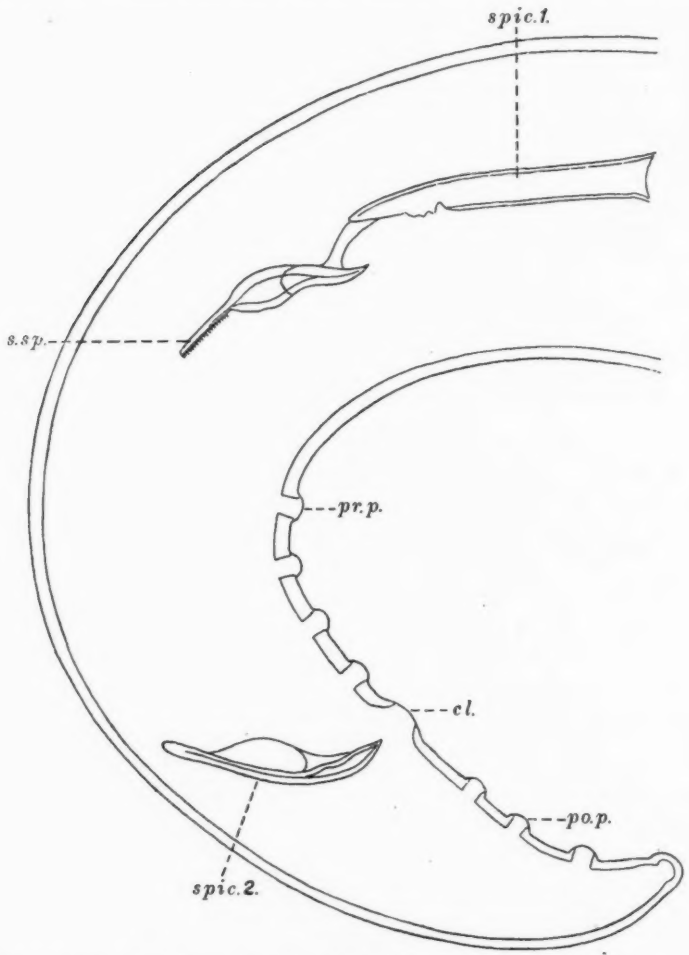

Fig. 7. *Setaria Hornbyi* sp. n. Lateral view of caudal extremity of male. × 220.

fold and in the peritoneal cavity. Mr Hornby writes that he has seen similar forms parasitic in the Eland, Water-Buck and Reed-Buck.

Specific diagnosis. *Setaria:* Anterior end of the body narrowing very gradually to a point about the level of the nerve-ring, in front of which it

tapers sharply to the oral extremity. The comparatively small head is separated from the rest of the body by a slight neck-like constriction (Text-fig. 5).

Peribuccal ring in the shape of a short oval, its dorso-ventral axis being only slightly longer than the lateral; dorso-ventral and lateral notches of approximately equal depth so that the buccal armature gives the impression of a shallow ring with four truncated tooth-like processes projecting anteriorly in the submedian planes (Text-fig. 6).

Submedian and lateral head-papillae small.

Anterior and posterior regions of the oesophagus measure 0·63–0·7 mm. and 9·5–10·3 mm. respectively.

Male: 108–110 mm. in length with a maximum breadth of about 0·5 mm.

Tail region narrow, coiled in a close spiral and terminating in a rounded knob. Lateral caudal appendices were not observed and are probably absent in this species (Text-fig. 7).

Cloaca about 0·28 mm. from the posterior extremity. Four pairs of preanal and three pairs of postanal papillae.

Spicules very unequal, the longer having a length 0·45–0·47 mm. and consisting of an anterior cylindrical region, 0·25–0·27mm. long and a posterior partly membranous region the terminal portion of which is finely serrated (Text-fig. 7).

Female: 200–260 mm. in length, the body having a maximum thickness of 1·25–1·5 mm.

Anus 0·8 mm. from the posterior extremity which is provided with a sucker-like terminal appendage (Text-fig. 6). A pair of small lateral appendices are situated 0·05–0·08 mm. from the extremity.

Vulva 0·7 mm. from the anterior end of the body.

REFERENCES.

LINSTOW, O. VON (1906). Helminths from the Collection of the Colombo Museum. *Spolia Zeylan.*, III. 163–188.

RAILLIET, A. et HENRY, A. (1911). Sur une Filaire péritonéale des Porcins. *Bull. Soc. Path. exot. Paris*, IV. 386–389.

—— (1911). Remarques au sujet des deux Notes de MM. Bauche et Bernard. *Bull. Soc. Path. exot. Paris*, IV. 485–488.

STILES, C. W. (1907). The zoological characters of the Roundworm genus *Filaria* Mueller 1787, with a list of the Thread Worms reported for Man. *Bull.* 34, *Hyg. Lab., U.S. Pub. Health and Mar. Hosp. Serv.*, Washington, 31–51.

STOSSICH, M. (1897). Filarie e Spiroptere. Lavoro Monografico. *Boll. Soc. adriat. di Sci. nat. in Trieste*, XVIII. 13–162.

EXPLANATION OF LETTERING TO TEXT-FIGURES.

a.—anus of female; *c.a*—caudal appendices; *cl.*—cloaca of male; *l.*—lateral lip of peribuccal ring; *n.r.*—nerve-ring; *oes.*1.—anterior region of oesophagus; *oes.*2.—posterior region of oesophagus; *p.r.*—peribuccal ring; *po.p.*—postanal papilla of male; *pr.p.*—preanal papilla; *s.p.*—submedian head-papilla; *s.sp.*—serrated end of spicule; *sp.*—ring of caudal spines; *spic.*1.—long spicule of male; *spic.*2.—short spicule; *t.*—dorsal tooth of peribuccal ring; *te.*—terminal knob of caudal region; *v.*—vulva.

OBSERVATIONS ON THE INTESTINAL PROTOZOA OF THREE EGYPTIAN LIZARDS, WITH A NOTE ON A CELL-INVADING FUNGUS.

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(With Plates XIX and XX, and 2 Text-figs.)

While I was in Egypt conducting investigations into the etiology of dysentery with Captain F. W. O'Connor at Alexandria in 1916 I was able to make some observations on the intestinal protozoa of three species of lizard. The occurrence of an *Entamoeba* and a *Chilomastix* (*Tetramitus*) in one of these, *Agama stellio*, was mentioned in our publication on the *Human Intestinal Protozoa in the Near East*, p. 147 (1917). The other lizards investigated were *Chamaeleon vulgaris* and *Lacerta agilis*. I will describe the observations on the protozoa under their respective hosts.

1. *Chamaeleon vulgaris.*

The only protozoon found in the gut of the chamaeleon was a flagellate of the leptomonas type and it was confined almost entirely to the cloaca, though sometimes it extended in small numbers into the rectum. The only previous record of such a flagellate in the cloaca of the chamaeleon is in a paper by Bayon (1915), who discovered it in *Chamaeleon pumilus* in Robben Island in 1914. I had the opportunity of examining Dr Bayon's preparations and there can be no doubt that the measurements he quotes are erroneous. The length of the flagellate bodies is given as 25–75μ; breadth 2–10μ; diameter of trophonucleus 2·5–4μ. In none of his preparations did I see flagellates of this size, nor have I found them in the parasite of the Egyptian *Chamaeleon vulgaris*. Evidently some mistake in calculation was made and the measurements are at least three times what they should be.

Structure of the flagellate.

The organism (Pl. XX, Fig. 1) as it occurs in the Egyptian lizard has the usual leptomonas structure—an elongate, flattened and pointed body, a central nucleus and a terminal and anterior kinetoplast with a long flagellum directed forwards, in which direction the flagellate progresses. The kinetoplast, which in many individuals is laterally placed (Fig. 1, *o*), as in other organisms of this

group, consists of a dark-staining granule, the parabasal, in front of which is a paler and smaller granule, the blepharoplast or centrosome from which the rhizoplast, continued into the flagellum, actually arises. Between these two granules, and also frequently behind them, is a clear area. Whether the whole structure represents a true nucleus of which the dark granule is the karyosome and the limits of the clear area a nuclear membrane on which the centrosome lies, as in the nucleus of *Cercomonas*, is still a matter requiring elucidation. I have brought forward some evidence in favour of the true nuclear nature of this structure in a paper entitled "Observations on *Herpetomonas muscae domesticae* and some Allied Flagellates" (1913). Most observers, however, seem to regard the structure as not being a nucleus and I quite agree there are many arguments against this view. Kofoid (1915) has suggested the abandonment of the name kinetonucleus and with it the binucleate conception of this group of flagellates. According to him the darkly staining granule is a parabasal body, a term first used by Janicki (1911), and homologous with similar structures which are associated with the flagellar origin in many other flagellates (*Trichomonas, Chilomastix, Giardia*, etc.) and which occurs in the flagellate *Prowazekella lacertae* considered below. The blepharoplast is the centrosome and leads the way in division. It must be admitted, however, that in division the behaviour of the centrosome and the subsequent division of the darkly staining mass by elongation and constriction, evidently under the influence of the centrosome, bears a striking resemblance to the division of the trophonucleus of these flagellates or of the nucleus of *Cercomonas*, as I have shown elsewhere (1913). Whether there is a nuclear membrane or not surrounding the clear area in which the darkly staining granule lies is largely a matter of interpretation, for one is dealing with such tiny objects that it is exceedingly difficult to determine this point with accuracy. In such a decision one is naturally influenced by what one has observed in larger flagellates. On account of the doubt surrounding the true nature of this structure it is better to avoid any term implying a nuclear nature so I have adopted the name kinetoplast, first suggested, I believe, by Alexeieff. The use of this word avoids the necessity of such ponderous names as kinetonucleus and trophonucleus. The kinetoplast includes both the parabasal and the blepharoplast. In degenerate trypanosomes in which the cytoplasm has disappeared the kinetoplast may remain as a compact body showing the parabasal surrounded by what appears to be a membrane on which the blepharoplast lies. The flagellum may or may not remain still attached to the blepharoplast.

As a rule the cytoplasm of the organisms is quite clear. In some, however, darkly staining granules are present, but these are probably dependent upon the state of nutrition.

The length of the body of the largest individuals (Pl. XX, Fig. 1, *f* and *m*) is about 15 μ and the flagellum is slightly longer than this. The width of the body of these long forms is under 3 μ. From these there may be traced a series of gradually diminishing individuals of a great variety of shape and size, as shown

in Fig. 1. Finally, very minute forms (Fig. 1, *a* and *b*) more or less spherical or circular in outline are seen, having a diameter of about 2 μ. These minute flagellates have relatively long flagella. Some of them are devoid of flagella (Fig. 1, *b* and *e*) and from them small ovoid bodies with a more definite outline appear to arise (Fig. 1, *d*). These are probably encysted forms such as occur in the similar flagellate of the flea, *Pulex irritans*. I have shown in this case that complete drying of the spread-out flea faeces for 24 hours does not prevent a culture being obtained when introduced into N.N.N. medium (1912). Between these tiny parasites and the elongated form every intermediate stage can be easily traced (Fig. 1, *g*, *i*, *j*, *k*). In the small forms the nucleus and kinetoplast lie close together.

Multiplication takes place in the usual manner by longitudinal fission after division of the nucleus and kinetoplast. The new flagellum is formed by an outgrowth from the daughter blepharoplast (Fig. 1, *h*, *l*, *n*).

I have been quite unable to make out any connexion between the kinetoplast and the nucleus, nor have I seen any indication of an axostyle.

Distribution of the flagellate.

The flagellate was found in all the chamaeleons examined—about six. As already stated, it was confined almost entirely to the cloaca into which the rectum and ureters open. It was encountered in small numbers in the rectum, but not in the ureters, while in the cloaca itself it was limited almost entirely to the surface of the mucosa where it occurred in the mucus in enormous numbers. When the lizard defecates it passes a cylindrical mass of faeces or solid white urine which is covered with this mucus in which swarms of flagellates can be found. In the actual urine itself they do not occur and in the faeces only in small numbers. It is probable that the latter are forms which have passed down from the rectum. The mucus from the cloaca is a clear transparent substance and is remarkable in that there is practically no contamination with bacteria or with faecal material. In stained smears no bacteria were seen and the only organism met with apart from the flagellate was the fungus described below, which was present on two occasions.

Careful examinations of the various organs of the body both by smear and cultural methods failed to reveal any tissue infection. This is perhaps surprising in the light of the work of the Sergents, Lemaire and Senevet (1914) and the later work of Chatton and Blanc (1918). These observers have shown that cultures of leptomonas can be obtained by inoculating N.N.N. medium with the blood of the gecko, *Tarentola mauritanica*. Examinations of smears of the blood and organs failed to reveal any leishmaniform parasites, the flagellates only being demonstrated by the culture method. It seems probable that the leishmaniform bodies seen by Chatton and Blanc (1914) within the red blood corpuscles of the gecko on an earlier occasion have no connexion with the leptomonas obtained by them later in cultures. In the light of these successes it would be interesting to make further attempts to isolate a lepto-

monas from the blood and tissues of the chamaeleon, for it would seem that in this animal the intestinal infection may indicate the path by which the blood infection of the gecko was acquired.

The exact relation of the flagellates of the chamaeleon to the mucosa is best studied in sections. It is found that the infection is a very large one and that all the glands of the cloacal mucosa have their ducts packed with the organism (Pl. XIX, Fig. 1). No trace of invasion of the cells could be found, so it would appear that the flagellate is limited to the ducts of the glands and the surface of the mucosa. Fig. 2 shows a single gland more highly magnified and though many goblet cells are present there is no tendency for the flagellates to make their way into these. There was no evidence that the flagellate in any way inconvenienced its host.

Source of the infection.

As was suggested by Bayon (1915), the most probable source of infection would appear to be some insect which the chamaeleon has eaten. Flies could very readily infect themselves by feeding on the mucus covering the faeces passed by the chamaeleon, which in its turn could be infected by eating an already infected fly. Such a hypothetical cycle would seem to be the probable one.

The contents of the stomachs of chamaeleons were examined with a view to identifying the flies on which they had fed, but little information was obtained, chiefly through lack of time to follow the observations properly. However, some experiments were conducted with the ordinary house-fly, *Musca domestica*. Pupae were collected and it was found that the flies hatching from these were free from flagellate infections. A batch of these hatched flies was fed on the infected mucus from the chamaeleon's cloaca and kept alive by feeding on sugar and water. Another batch was used as control and kept alive in a similar manner.

24. VI. 16. Flies fed on infected mucus.
25. VI. 16. Several flies examined.
    1. Stomach with large infection of very active flagellates.
    2. Ditto.
    3. Active flagellates in stomach and rounded forms in intestine.
    4. Active flagellates in both stomach and intestine.
    5. Doubtful forms seen.
    6. Ditto.
    7. Rounded forms in intestine.
    8. Active flagellates in stomach and round form in intestine.
    9. Ditto.
    10. Active forms in stomach and intestine.
26. VI. 16. One fly had resting forms in intestine.
30. VI. 16. Four flies all negative.
1. VII. 16. Thirteen flies all negative.

None of the flies in the control experiment showed any infection.

It is evident therefore that the flagellates taken up by the flies can survive for at least two days, and the character of the infections in the flies resembled a natural fly infection, but the fact that the flagellates eventually disappeared would seem to indicate that the house-fly is not the true host of the chamaeleon flagellate.

Unfortunately time did not permit of the investigations being carried any further.

Nomenclature.

The flagellate of the chamaeleon is undoubtedly of the leptomonas type and it is of especial interest as being the only known leptomonas which is parasitic in the gut of a vertebrate. In addition to the flagellates of the gecko described by the French observers others have been recorded from vertebrates, apart of course from the well-known natural leishmania infections of man, dog and cat. The Sergents (1907) recorded their observation of a leptomonas in the blood of the pigeon. Fantham and Porter (1915) say they saw a leptomonas in mice but Mesnil (1915) is doubtful of the correctness of this observation. Balfour (1916) also states that he and Archibald saw such a flagellate in the gerbil in the Sudan. Dutton and Todd (1902) claimed to have seen this flagellate in Gambian house mice (sp. ?), but in a subsequent examination of the films Todd (1914) found the flagellate to be in reality a trypanosome which he identified with *Trypanosoma acomys* (Wenyon). *Trypanosoma lewisi* is a very active flagellate and occurs in such a variety of forms that when seen only in the fresh unstained blood it is quite easily mistaken for a leptomonas and it seems probable that the forms described by the various observers noted above were in reality *Trypanosoma lewisi*. In any case the descriptions are so meagre that it is impossible to identify the flagellates. In 1919 Marcel Leger described a flagellate (a leptomonas) which he named *L. Henrici* from the blood of two out of thirty lizards (Genus *Anolis*, Fam. Iguanidae) examined in Martinique. The organisms were typical leptomonas forms measuring 15–16μ in length and 3–4μ in breadth. The flagellum was longer than the body. Rarely were rounded leishmania forms seen. He subsequently found that over half the lizards examined harboured what was apparently the same leptomonas in the rectum. It would appear, therefore, that the only cases of leptomonas infections of the blood and tissues of animals are those of the N. African gecko and the S. American *Anolis*. In 1909 Knuth recorded the finding of a leptomonas in the heart blood of a roebuck but, as the animal was partly devoured and had fly larvae in its lungs, the infection may have been of extraneous origin.

On the other hand, it has been demonstrated, chiefly by Laveran and Franchini and Fantham and Porter, that many insect flagellates of the leptomonas and crithidia types are inoculable into mice and other animals and even bring about their death. These inoculation experiments are of the greatest interest from the point of view of the spread of leishmaniasis, and the French

observers (1914) have suggested that the flagellate of the gecko may in reality be *Leishmania tropica*, and that it is transmitted to man by the *Phlebotomus* which feeds on the lizards. On account of their importance it would be well if the inoculation experiments were repeated by other observers.

In the present state of our knowledge it is difficult to name many of these flagellates. Some time ago (1913) I outlined a scheme for the classification of these flagellates in the following manner. There is a group, confined entirely to the insect or invertebrate host, which in its most highly developed stage has the leptomonas structure. Small round leishmaniform bodies, protected by what must be a cyst wall, are developed from the leptomonas forms and escape in the insect's faeces. They are ingested either by the larvae or adults of the insect and lead to their infection. There is thus only a single invertebrate host and such forms can be distinguished, at any rate at present, by the generic name *Leptomonas*. This name was first employed by Kent (1881) for a flagellate seen by Bütschli in 1878 in a nematode worm *Trilobus gracilis*. Kent named it *Leptomonas bütschlii*, but whether it is a leptomonas as we now understand it cannot be determined till this organism is re-examined in the light of present knowledge. Meanwhile we shall employ the name *Leptomonas* as above defined.

Another group of flagellates, in which the highest stage of development is again the leptomonas form, includes the parasites of leishmaniasis. Here, however, there is a vertebrate host in which the leishmaniform parasite is most usually seen, but also occasionally the leptomonas, as I have shown (1915). The latter is generally encountered only in cultures in the test-tube or in certain invertebrates which have ingested the leishmania forms along with a quantity of blood. There is undoubtedly an invertebrate host of this flagellate and in it the infection would be expected to resemble a true leptomonas of an invertebrate. In order to distinguish these flagellates which have a vertebrate as well as an invertebrate host from the purely insect form—the true *Leptomonas*—the generic name *Leishmania* can be employed.

A third group of flagellates attains a still higher stage of development and the individual flagellate is known as a crithidia. In it the kinetoplast is close to, but still in front of, the nucleus, and there is a short undulating membrane running from just in front of the kinetoplast to the anterior end of the flagellate. Along the edge of this runs the flagellum, to become free at the anterior extremity of the flagellate. The crithidia live in the intestine of invertebrates and in the posterior part of the intestine there are produced small leishmania forms, as in the leptomonas, and they are responsible for the spread of infection from host to host. There is again only a single host—an invertebrate—and such forms may be considered under the generic name *Crithidia*. We know of no flagellate of this type which has both a vertebrate and an invertebrate host corresponding to the *Leishmania*.

A fourth group shows a still higher development in that the true trypanosome structure is attained. The kinetoplast is further back at the posterior

end of the body and there is a long undulating membrane along which the flagellum runs, to become free at the anterior extremity of the flagellate. In the insect's intestine these forms show not only the trypanosome type but also intermediate ones—crithidia and leptomonas—and finally the leishmania forms. In flagellates of this group there is again only a single invertebrate host and the infection is spread from insect to insect by the leishmania forms as in *Leptomonas* and *Crithidia*. A flagellate of this group is *Herpetomonas muscae domesticae* of the common house-fly and it is convenient to distinguish these forms by the generic name *Herpetomonas*.

In a fifth group there are the same forms as in the preceding group, but in place of the single invertebrate host there is a vertebrate one as well. This is the group of true trypanosomes which have the generic name *Trypanosoma*.

This scheme of classification may have many points against it, but at any rate it is convenient and is as far as we can go in our present state of knowledge. To subdivide these groups into different genera simply leads to further confusion. All we can do is to wait for further data, and such an attempt as that made by the late Dr Albert Chalmers (1918) to split up the genus *Trypanosoma* into his extraordinary series of new genera only tends to bring confusion into an already difficult subject.

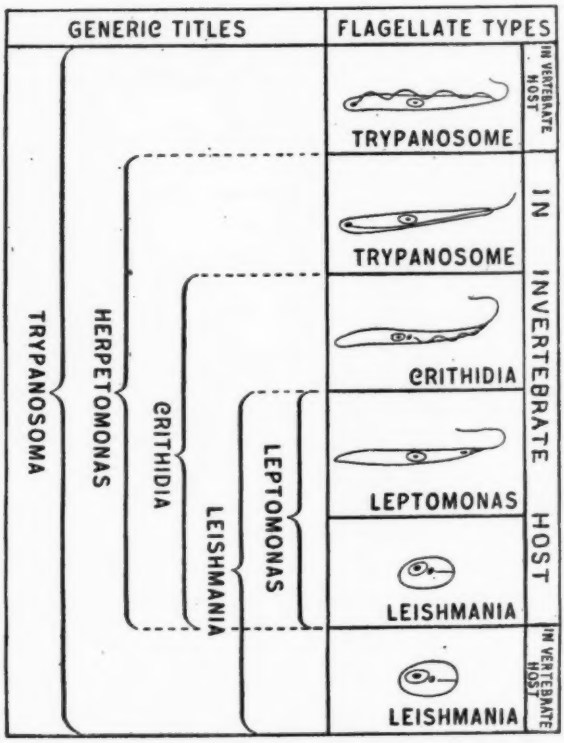
The scheme I have outlined above has the merit of simplicity and it can be arranged in tabular form in the following manner. The names in the right-hand column (Text-fig. 1) are merely descriptive and should be used in an adjectival sense. They may be employed for any particular stage which may appear in the development. Those in the left-hand column are generic titles and, as such, should be written in italics and with a capital. We can speak, for instance, of the leptomonas stage of *Leishmania tropica*, the crithidia stage of *Trypanosoma lewisi*, or the leishmania stage of *Herpetomonas muscae domesticae*.

There is one group of flagellates which do not enter into the above scheme. I refer to the curious leptomonas form first described by Lafont from the latex of Euphorbias as *Leptomonas davidi*. The work of Lafont, Bouet and Roubaud, and more recently of França (1919), has demonstrated that this flagellate undergoes a cycle in a hemipteron and is transmitted by it to healthy plants. Thus we have a flagellate of the leptomonas type which has both an insect and a plant host. Evidently it cannot be included in any of the genera defined above. For these flagellates we may employ the name *Phytomonas*, first suggested by Donovan (*Lancet*, 1909), the type species being *Phytomonas davidi* (Lafont, 1909).

If the flagellates of this most confusing group were named according to the scheme outlined above we should at any rate understand from their names something of their life history and structure.

The question now arises into what group we are to place the flagellates of the gecko, the anolis and the chamaeleon. I think we are safe in assuming that there must be invertebrate hosts. The two former would undoubtedly

fall into the group leishmania, according to the definition given above, and would receive the provisional names of *Leishmania tarantolae* and *Leishmania Henrici*. If it should subsequently be found that the flagellate of the gecko is in reality identical with that causing Oriental sore then it would of course become *Leishmania tropica*, but proof of this is at present wanting. The flagellate of the chamaeleon occupies an intermediate position. It undoubtedly has an insect host but, unlike the parasite of the gecko, it is not a tissue parasite and as far as my investigations go it appears to be confined to the



Text-fig. 1. Diagram of classification of the trypanosomes and allied flagellates.

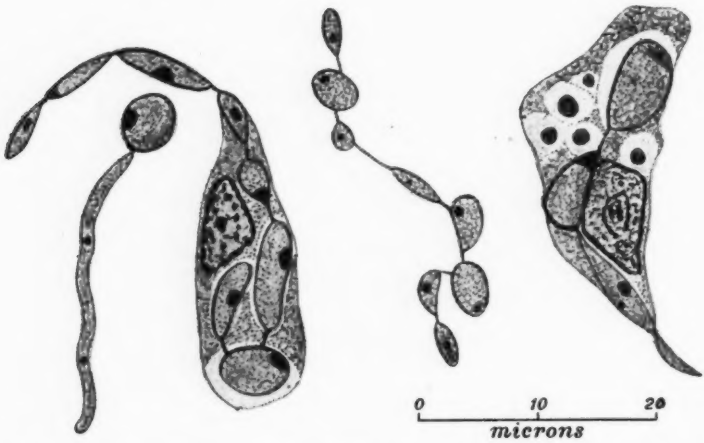
intestine and cloaca. However, it comes within the definition as having both hosts and it will therefore become *Leishmania chamaeleonis*. It seems better to place it in the genus *Leishmania* than in *Leptomonas*, for the latter would imply that it was a purely insect, or at least invertebrate, flagellate.

The intestinal habitat is of interest as it is an infection probably acquired from some insect and indicates a stage by which such an insect flagellate may eventually become a tissue parasite. It occupies an intermediate position between the purely insect flagellate and the vertebrate ones such as that of

the gecko, and of Oriental sore and kala-azar. Moreover it suggests the possibility of an intestinal mode of infection in the latter disease. In the case of *L. Henrici* it appears as if the intestinal infection, still persisting in the lizard, has already given rise to a blood and tissue infection.

Note on a cell-invading fungus.

In some of the chamaeleons examined it was noted that a fungus (Text-fig. 2) was present, and in smears and sections it was found that there was a definite invasion of the cells of the cloaca. The fungus occurs in the form of ovoid bodies embedded in the cytoplasm of the cells. Reproduction takes place by budding till the whole cell is invaded. In this process the nucleus becomes pushed aside and distorted and the cell eventually degenerates. Buds are



Text-fig. 2. The fungus which was found invading the cells of the cloaca of *Chamaeleon vulgaris*. *C. M. W. del.*

also formed, protruding into the cavity of the cloaca and when this has occurred growth into the lumen takes place with the formation of more elongate elements and finally filaments. No culture of this organism was attempted and nothing more of its life-history is known. The general characters are depicted in the drawing shown in Text-fig. 2.

2. *Lacerta agilis* and *Agama stellio*.

The protozoal organisms seen in the gut of these two lizards resembled one another and I have no reason to suppose that the forms common to these two lizards belong to different species. Those common to the two are *Bodo lacertae*, Grassi, or, as Alexeieff (1912) has renamed it, *Prowazekella lacertae*, *Chilomastix* sp., and *Entamoeba* sp. In addition to these three organisms, in

Agama stellio was also found a trichomonas and a trichomastix. These are probably identical with *Trichomonas lacertae*, Prowazek, and *Trichomastix lacertae*, Bütschli. It is possible that if a number of *Lacerta agilis* had been examined they would have been found in them also. The Entamoeba may possibly be identical with one seen by Dobell (1914) in the gut of *Lacerta muralis*.

Prowazekella lacertae Grassi.

This flagellate has been the subject of some controversy on account of its association in the gut of the lizard with a blastocystis, or structures which have a striking resemblance to this. Prowazek (1904) claimed to have demonstrated that the blastocystis was in reality the autogamy cyst of the flagellate. It is very improbable that any autogamy takes place in the life-history of the flagellate, but nevertheless it is undoubtedly true that the flagellate encysts and that the cysts produced have frequently been spoken of as blastocystis. Chatton (1917) describes the blastocystis as a stage in the developmental cycle of the flagellate and finds that under certain conditions not well understood the large blastocystis resulting from growth of the smaller forms produces large numbers of flagellates. He supposes, and probably correctly, that a conjugation between the flagellates takes place in association with the encystment.

Description of flagellate.

The flagellate (Pl. XX, Fig. 3) is in its adult stage an elongate, flattened organism with two tapering flagella, one of which is directed forwards and is several times the length of the body, and the other, a thinner and shorter one, directed backwards. There is a single anterior nucleus, on the anterior surface of the membrane of which lies a granule from which the flagella arise.

The body of the flagellate, as already mentioned, is flattened like a blade of grass, but not perhaps to the same extent, and sometimes, as noted by Prowazek (1904), the edges are folded or the body may be twisted on itself, but as usually seen it is simple in structure with the posterior end pointed and tapering to a varying extent. The anterior end is also pointed but less acutely than the posterior end. The cytoplasm is vacuolated and may contain granules. Near the anterior end lies the spherical nucleus, which is closely surrounded by a number of deeply staining bodies. In iron haematoxylin preparations these tend to obscure the nucleus unless the differentiation is carried far enough. The nucleus itself is a spherical body consisting of a nuclear membrane and a centrally placed karyosome. Fine granules may also be seen within the membrane, either on its inner surface or between it and the karyosome. On the anterior surface of the membrane is the granule from which the rhizoplast arises. This granule does not retain the stain as intensely as the karysosome of the nucleus nor the bodies which surround the nucleus. In some cases it appears that the granule may be double but it is difficult to make out whether this is merely an early stage of division or not. From the

granule there runs forward a fine rhizoplast. The deeply staining bodies which surround the nucleus vary considerably in number and arrangement. Sometimes they are closely applied to the nuclear membrane while at others they are separated from it by a distinct interval. There may be many fine granules or a few larger masses. They may surround the nucleus or be limited to one side only or be entirely behind it. There may be only two bodies, one on each side of the nucleus, and of such a shape that they are swollen posteriorly and tapering anteriorly, with their anterior extremities nearer one another than the posterior ones. Prowazek (1904) notes these bodies and regards them as composed of chromatin material derived from the nucleus. I can find no evidence that these bodies arise from the nucleus. In those cases where they appear to be within the membrane careful observation shows that they are merely overlying it. Prowazek (1904) figures forms in which these bodies are on the inner surface of the membrane but in all the forms I have seen they are outside it even if lying against it. Prowazek's idea that they are chromidial in nature was first contested by Dobell (1908), who wrote: "I think sufficient has been said to show that autogamy and chromidia are as yet unproven in the case of Bodo." They can best be spoken of as parabasal bodies similar to those which are associated with the origin of flagella in other flagellates.

The rhizoplast arising from the blepharoplast or centrosome on the nuclear membrane passes forwards and is continued into the two flagella. At the extreme anterior end of the organism at the point where the flagella arise from the rhizoplast is occasionally seen a granule or thickening but I have been unable to make out any structure in this (Pl. XX, Fig. 3, *a*, *c*, *g*). Prowazek (1904) figures the rhizoplast as terminating near the anterior end of the body in a sort of cone, beyond which is a second granule from which the flagella actually arise. If this structure is really present and there is a break in the rhizoplast then the flagellar origin is of a very specialized type. Is it not possible that this appearance is due to some peculiar plasticity of the anterior end of the body, which by retraction at the point of exit of the flagella gives rise to the cone-like appearance? At any rate in my preparations many of the flagellates seem to have a rhizoplast continued directly from the blepharoplast into the flagella. I have been unable to trace any connexion between the blepharoplast and a granule within the karyosome of the nucleus such as Prowazek describes.

The anteriorly directed flagellum is often at least five times the length of the body of the elongate flagellates and in some of the smaller and ovoid forms it is even longer in proportion. The posteriorly directed flagellum reaches as much as three times the length of the body in the long forms. It is very much finer than the anteriorly directed flagellum and in some individuals it appears to be attached to the body for a short distance (Pl. XX, Fig. 3, *a*, *c*, *e*), an attachment which may have to do with maintaining its direction. In many of the flagellates, however, there is no such attachment.

Multiplication.

The flagellate multiplies in two ways, either by simple division or cyst formation. I have not been able to trace the division in much detail, as dividing flagellates were not numerous in my preparations. However, evident dividing forms were seen in which two nuclei were present, each with its blepharoplast and rhizoplast passing into two flagella. The parabasal bodies were divided between the two nuclei so that in nuclear division those of the original nucleus are evidently divided into two more or less equal groups, as occurs in nuclear division within the cyst.

Encystment takes place in the hinder part of the gut of the lizard and is preceded by a change in shape of the organism. It becomes an ovoid body and apparently two of these become encysted together (Pl. XX, Fig. 3, *n*). A feature of the encystment is that clumps occur, the individuals of which are all in approximately the same stage of development. The clump appears to be held together by an adhesive material in which various bacteria and other intestinal debris are included.

The first stage is the formation of the ovoid body, which loses its flagella. Two of these come together and a cyst wall is formed round the pair (Fig. 3, *j* and *o*). The cyst wall is evidently of a gelatinous nature, for bacteria and other debris adhere to it. Within the cyst the two organisms fuse, as noted by Prowazek (1904), and this, I believe, is followed by fusion of the nuclei (Fig. 3, *p–s*). I can see no evidence of an autogamy as described by him.

The next stage is the appearance of a vacuole in the cytoplasm of the zygote and this gradually increases in size, evidently by absorption of fluid through the cyst wall, till the cyst becomes many times its original bulk. Concurrently with this vacuolation nuclear division takes place (Fig. 3, *t–y*). The original nucleus of the zygote has the same structure as that of the flagellates, being spherical with a nuclear membrane and central karyosome (Fig. 3, *r* and *s*). The parabasal bodies lie around the nucleus. The exact similarity between the nuclei of the cyst and the flagellates makes it practically certain that they are in reality flagellate cysts, quite apart from the stages of encystment where every step can be followed. In division the karyosome divides and the two parts separate, while they remain connected by a fine fibre (Fig. 3, *u–w*). The nuclear membrane elongates at the same time, and finally two nuclei are formed by constriction at the middle. The parabasal bodies remain at the equator of the elongating nucleus for some time and then they are divided into two groups which pass to the daughter nuclei. These bodies are of various sizes and they do not appear to form anything in the nature of chromosomes. I cannot say if any actual division of each separate mass takes place but each of the resulting groups of daughter parabasal bodies contains more or less an equal amount of material. The nuclei continue to divide in the same manner till as many as 32 are formed within the now very enlarged cyst (Fig. 3, *m, l, i*). The majority of the cysts are spherical but some are

elongate or even dumb-bell shaped. The growth of the cyst is remarkable but a similar growth takes place during the development of the oöcyst of the malarial parasite in the stomach of the mosquito. It is evident that the cysts are not very resistant bodies, but that they are destined to pass out of the body is supported by their occurrence in greatest number at the hinder end of the intestine.

The fully formed cyst contains comparatively little cytoplasm, which is grouped around the nuclei on the inner surface of the cyst wall. The bulk of the cyst contains liquid, through which strands of a coarse network of more refractile material run and which connect with the nuclear areas. Even though not possessed of very tough or impermeable walls it is possible that these cysts would take a considerable time to dry completely on account of their enormous fluid content. The development of the cyst beyond the 32 nuclear stage I have not been able to follow but Prowazek (1904) and later Chatton (1917) have seen these large cysts give rise to numbers of flagellates.

The description I have given depends entirely on stained films as prolonged observations on the living cysts were not made. That two individuals encyst together seems undoubted but if one wished to be hypercritical one could suppose that the two associated individuals either before or after encystment were the results of division and do not represent conjugation. My interpretation of the appearances seems the more probable one and is in conformity with Chatton's own observations. It must, however, be admitted that an absolute proof of the process has not been obtained by me. Further observations on this parasite might yield some interesting facts in connection with the conjugation process of flagellates and the origin of the parabasal bodies and centrosome.

Blastocystis.

As already pointed out, many of these flagellate cysts bear a striking resemblance to blastocystis and as a matter of fact I have been accustomed to regard them as such. In the lizards I have examined, all the so-called blastocystis have the very characteristic nucleus with the surrounding parabasal bodies, so there can be no doubt that they are stages in the development of the flagellate cysts. Whether side by side with the true flagellate cysts there exists a "Blastocystis" of vegetable nature I cannot say at present, but it cannot be doubted that *Prowazekella lacertae* encysts in the gut of the lizard and that the cysts have been frequently styled "blastocystis."

What then is the blastocystis which occurs so commonly in the human intestine and that of other animals? Prowazek (1911) maintained that they were cysts of *Trichomonas* but there is no evidence to support this view. Swellengrebel (1917) has suggested that they are degenerate forms of various intestinal protozoa, while Jepps and Dobell (1918) have noted that certain degenerate forms of *Dientamoeba fragilis* resemble dead blastocystis. I myself have, for want of evidence to the contrary, always regarded them as of a vegetable nature and this may be the case in spite of the resemblance to

the cysts of *Prowazekella lacertae*. For a similar reason I regarded the I-cysts as being probably vegetable organisms, but they are now known to be cysts of *Iodamoeba williamsi*. I had also noted (1910) that degenerating *Chilomastix mesnili* could assume appearances closely resembling blastocystis. Swellengrebel's (1917) conclusion is that blastocystis "is not the name of a zoological genus but of a peculiar form of degeneration to which representatives of different genera of intestinal protozoa may be liable." On the other hand future investigations may show that blastocystis is derived from amoebae and it must be admitted that the large binucleate cysts of *Entamoeba coli* with the large vacuole occupying almost the entire cyst bears some resemblance to binucleate forms of "blastocystis." Frequently in stained preparations containing small entamoebae, such as *Endolimax nana*, and blastocystis, it is possible to trace what might be regarded as a complete series of connecting links between a typical amoeba and a typical blastocystis and one is constantly tempted to adopt the view that the series traceable is a real one. Macfie (1915) regarded certain blastocystis associated with an entamoeba in the monkey *Cercopithecus petaurista* as cysts of the entamoeba. His proof of this, however, appears to be wanting and furthermore we know that the entamoebae of the monkey produce the typical entamoebic cysts with four or eight nuclei.

Alexeieff (1911) says that at one time he regarded the blastocystis of the lizard as derived from the lizard flagellate but that later observations have altered his opinion and that he has come to look upon blastocystis as a purely vegetable organism. He was largely influenced in this by the character of the development of a yeast (*Schizosaccharomyces octosporus*), and has suggested the name *Blastocystis enterocola* for the intestinal blastocystis.

Dobell (1908) in criticizing Prowazek's work on the autogamy cysts of *Bodo* compares them with very similar cysts he had seen in the gut of the frog and which he proved by germination to be of a vegetable nature. It appears to me that Prowazek's cysts, though not autogamy cysts as he describes them, are at any rate true cysts of the flagellate. At least this can be stated of some of those he figures.

It is evident therefore that there is a difference of opinion as to the true nature of blastocystis and we must await further information. It seems possible that under the name blastocystis three distinct structures have been confused: the true protozoal cysts like those of the lizard flagellates, vegetable organisms like the cysts Dobell studied in the frog or which Alexeieff saw in the case of his yeast *Schizosaccharomyces octosporus*, and thirdly, degenerate intestinal protozoa or even tissue cells, some of those in the human intestine belonging to the second group and others to the third.

Trichomonas lacertae Prowazek, and *Trichomastix lacertae* Bütschli.

I have nothing to add to the description of these two organisms as given by Prowazek (1904). I have seen none of the conjugation forms described by him. The flagellates are shown in Pl. XX, Fig. 2, *h* and *i*.

Chilomastix sp.

This is a small organism of the usual structure and in size corresponds with the two flagellates just mentioned. Its general appearance is shown in Pl. XX, Fig. 2, *g*.

Entamoeba sp.

The entamoeba is a large organism in its fully grown form (Pl. XX, Fig. 2, *a*). Smaller forms also occur and the general character of the organism is shown in Fig. 2, *a–f*. It will be noted that it bears a striking resemblance to *Entamoeba coli* and, like it, it feeds upon most of the contents of the lizard's intestine, frequently ingesting the flagellates or their cysts (Fig. 2, *b*). It produces an eight-nuclear cyst which again is not distinguishable from that of the human *Entamoeba coli* either in size or characters.

Of the lizard hosts mentioned above, judging from the contents of their intestines *Chamaeleon vulgaris* is entirely an insect feeder. This is true to a large extent of *Lacerta agilis*, though in it some vegetable matter is also present. *Agama stellio* feeds on insects but also largely on vegetable matter, as large pieces of leaves of plants and grass are to be found in the stomach. It is not surprising therefore that the two last named can infect themselves from vegetable matter contaminated by other infected lizards, while the chamaeleon only harboured the flagellate which it had probably acquired from some insect, which had in its turn taken up the flagellate from the faeces or rather cloacal mucus of the animal.

REFERENCES.

ALEXEIEFF, A. (1909). Les flagellés parasites de l'intestin des Batraciens indigènes. *C. R. Soc. Biol.* LXVII. 199.

—— (1911). Sur les "Kystes de *Trichomonas intestinalis*" dans l'intestin des Batraciens. *Bull. Sci. France et Belgique*, XLIV. 333.

—— (1911). Sur la nature des formations dites "Kystes de *Trichomonas intestinalis*." *C. R. Soc. Biol.* LXXI. 296.

—— (1912). Sur quelques noms de genres des Flagellés qui doivent disparaître de la nomenclature pour cause de synonymie ou pour toute autre raison. Diagnoses de quelques genres récemment étudiés. *Zool. Anzeiger*, XXXIX. 674.

—— (1916). Mitochondries chez quelques protistes. Mitochondries glycoplastes. *C. R. Soc. Biol.* LXXIX. 1072.

BALFOUR, A. (1916). On the occurrence of Herpetomonads (?) in gerbils. *Parasitology*, VIII. 260.

BAYON, H. (1915). *Herpetomonidae* found in *Scatophaga hottentota* and *Chamaeleon pumilus*. *Trans. Roy. Soc. South Africa*, V. Part I. 61.

BRUMPT, E. (1912). Colite à *Tetramitus mesnili* (Wenyon, 1910) et colite à *Trichomonas intestinalis* Leuckart 1879. *Blastocystis hominis* n. sp. et formes voisines. *Bull. Soc. Path. Exot.* V. 725.

CHALMERS, A. J. (1918). The classification of trypanosomes. *Jl. Trop. Med. and Hyg.* XXI. 221.

CHATTON, E. (1917). Les "Blastocystis" stades du cycle évolutif de flagellés intestinaux. *C. R. Soc. Biol.* LXXX. 555.

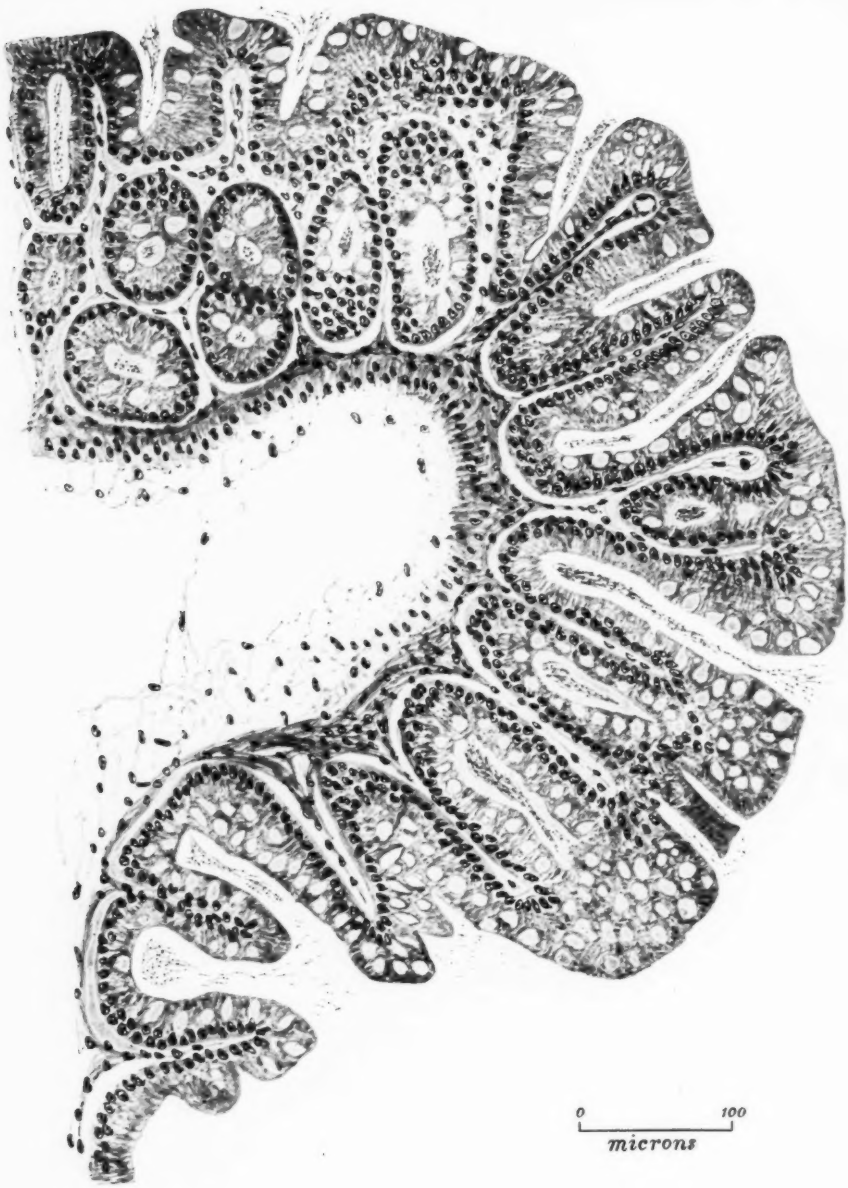


Fig. 1. Section through part of the wall of the cloaca of *Chamaeleon vulgaris* to show the lumen of the glands filled with clusters of *Leishmania chamaeleonis*. (*C. M. W. del.*)

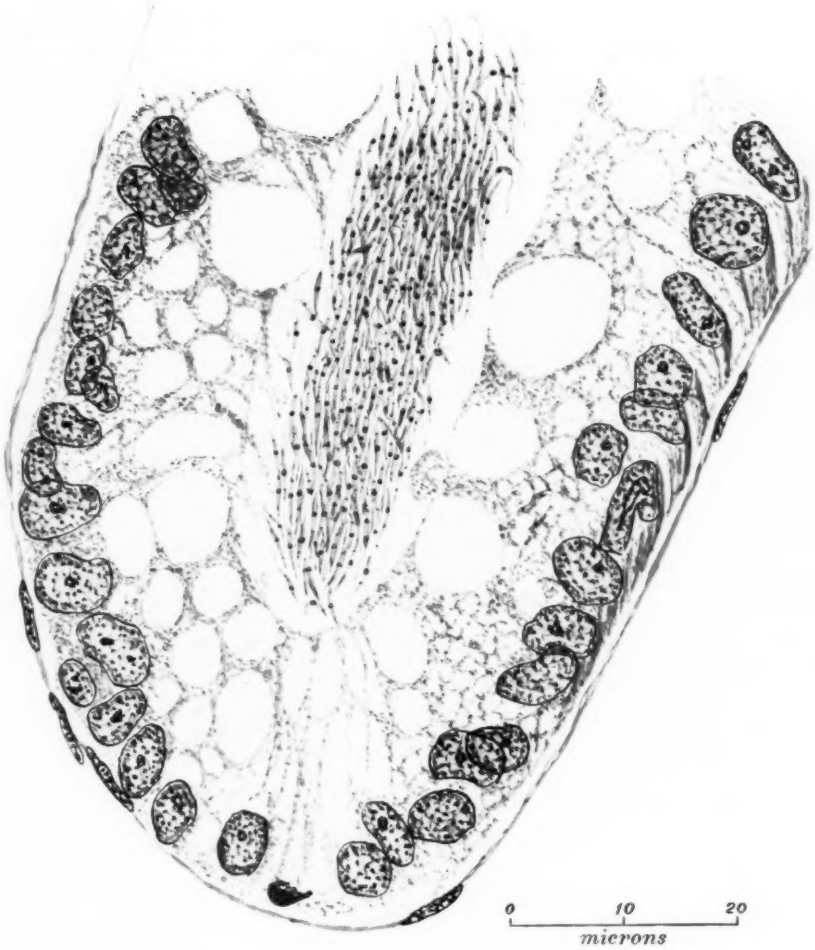


Fig. 2. A single gland in the same section as shown in Fig. 1 drawn to a larger scale. There appeared to be no invasion of the cells by the flagellates. (*C. M. W. del.*)

Fig. 1. Various types of *Leishmania chamaeleonis* found in *Chamaeleon vulgaris*. Every intermediate shape and size between the long forms (*f*, *l*, *m*, *n*, *o*) and the minute ones (*a*, *b*, *c*) are to be found. A possibly encysted form is shown at *d*. (*C. M. W. del.*)

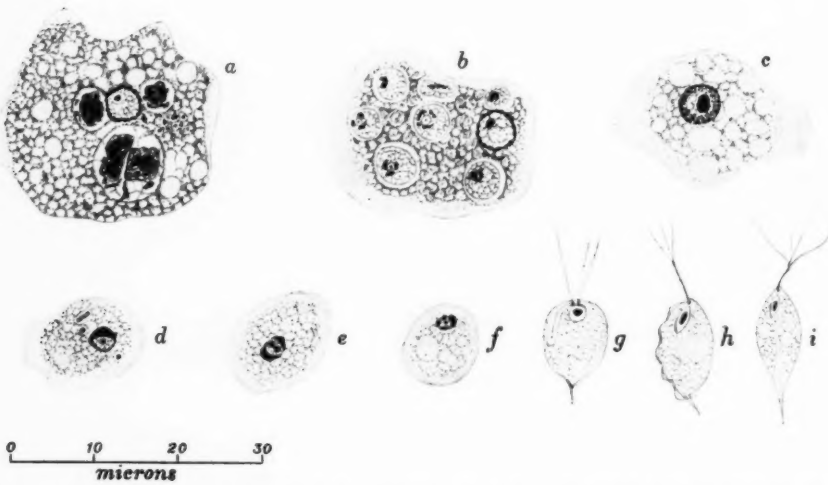


Fig. 2. Protozoa found in *Lacerta agilis* and *Agama stellio*. The entamoeba which produces cysts exactly like those of *Entamoeba coli* is shown at *a*—*f*; *Chilomastix* sp. at *g*; *Trichomonas lacertae* at *h*; *Trichomastix lacertae* at *i*. (*C. M. W. del.*)

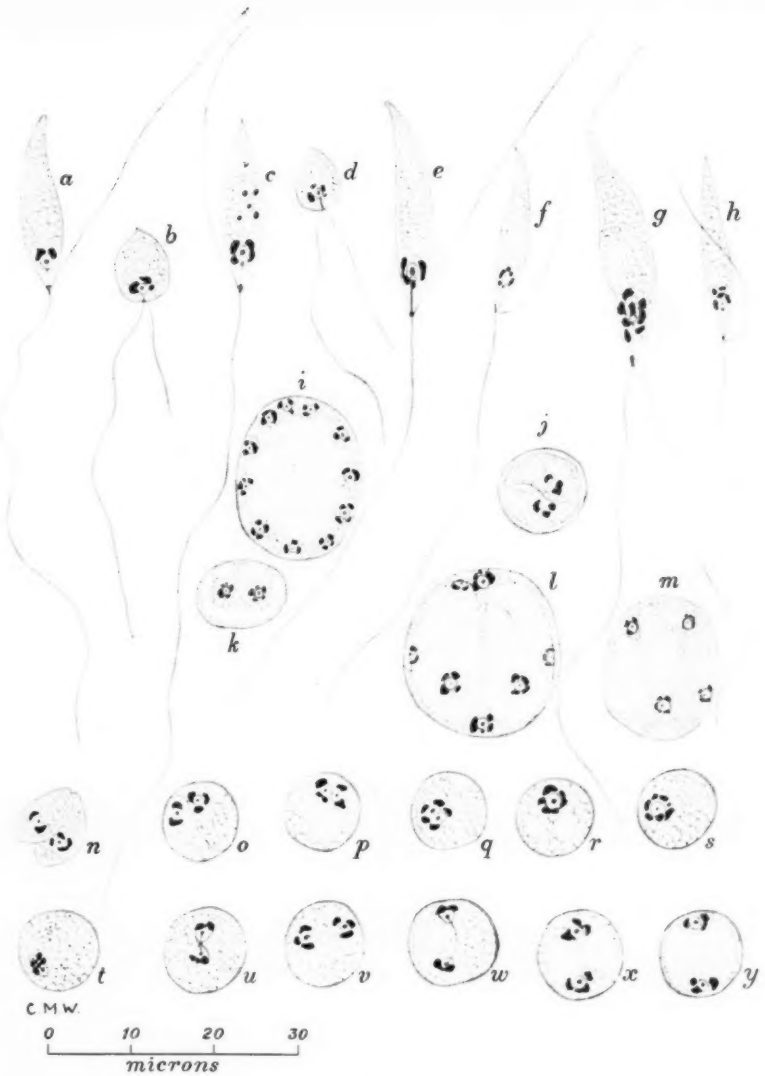


Fig. 3. *Prowazekella lacertae.* The forms figured at *a*, *c* and *e* show the appearance of the attachment of the "trailing" flagellum. In others both flagella appear to be quite free. The series *n* to *s* shows what is probably the conjugation and encystment, while *t* to *y* show the first nuclear division of the zygote and the formation of the vacuole. At *j* and *k* are two cysts corresponding to that at *o*. Later stages of the cyst development are shown at *m*, *l* and *i* in which nuclear multiplication to the 32 nuclear stage is reached. At all stages the parabasal bodies are seen surrounding the nucleus and they are roughly divided into two groups at nuclear division both of the free flagellates and the encysted forms. (*C. M. W. del.*)

CHATTON, E. et BLANC, C. (1914). Existence de corps leishmaniformes dans les hématoblastes d'un Gecko barbaresque, *Tarentola mauritanica*, L. Günth. *C. R. Soc. Biol.* LXXVII. 430.

—— (1918). Le *Leptomonas* de la Tarente dans une région indemne de Bouton d'Orient. Observations et expériences. *Bull. Soc. Path. Exot.* XI. 595.

CHATTON, E. et LALUNG-BONNAIRE (1912). Amibe limax (*Vahlkampfia* n. gen.) dans l'intestin humain. Son importance pour l'interprétation des amibes de culture. *Bull. Soc. Path. Exot.* V. 135.

DOBELL, C. C. (1908). Some remarks upon the "Autogamy" of *Bodo lacertae* (Grassi). *Biol. Centralblatt.* XXVIII. 548.

—— (1909). The "Autogamy" of *Bodo lacertae*. A reply to Dr v. Prowazek. *Biol. Centr.* XXIX. 363.

—— (1914). Cytological studies on three species of *Amoeba*—*A. lacertae* Hartmann, *A. glebae* n. sp., *A. fluvialis* n. sp. *Arch. für Protist.* XXXIV. 139.

DUTTON, J. E. and TODD, J. L. (1902). First Report of the Trypanosomiasis Expedition to Senegambia. *Liverpool School Trop. Med. Memoir XI.* p. 57.

FANTHAM, H. B. and PORTER, A. (1915). On the natural occurrence of Herpetomonads (Leptomonads) in mice. *Parasitology*, VIII. 128.

JANICKI, C. (1911). Der Parabasalapparat bei parasitischen Flagellaten. *Biol. Centralbl.* XXXI. 321.

JEPPS, M. W. and DOBELL, C. (1918). *Dientamoeba fragilis* n. g., n. sp., a new intestinal amoeba from man. *Parasitology*, X. 352.

KNUTH, P. (1909). Eine Herpetomonas beim Reh. *Zeit. Infekt. Krankh. Hausth.* VI. 357–362.

KOFOID, C. A. The biological and medical significance of the intestinal flagellates. *Second Pan-American Scientific Congress*, Washington, U.S.A., Dec. 27, 1915—January 8, 1916.

KUENEN, W. A. und SWELLENGREBEL, N. H. (1913). Die Entamöben des Menschen und ihre praktische Bedeutung. *Centralbl. f. Bakt. I Abt. Orig.* LXXI. 378.

LEGER, M. (1918). Infection sanguine par *Leptomonas* chez un saurien. *C. R. Soc. Biol.* LXXXI. 772–775.

MACFIE, S. (1915). A case of dysentery in a monkey in which amoebae and spirochaetes were found. *Ann. Trop. Med. and Paras.* IX. 507.

MESNIL, (1915). Review of paper by Fantham and Porter. *Bull. de l'Inst. Past.* XIII. 487.

PROWAZEK, S. (1904). Untersuchungen über einige parasitische Flagellaten. *Arb. a. d. Kais. Gesundheitsamte*, XXI. 1.

—— (1909). Zysten von *Bodo lacertae*. *Biol. Centr.* XXIX. 27.

—— (1911). Zur Kenntnis der Flagellaten des Darmtraktus. *Arch. für. Protist.* XXIII. 96.

SERGENT, ED. et ET. (1907). Etudes sur les Hématozoaires d'oiseaux. *Ann. Inst. Past.* XXI. 251.

SERGENT, ED. et ET., LEMAIRE, G. et SENEVET, G. (1914). Insecte transmetteur et Réservoir de virus du Clou de Biskra. Hypothèse et expériences préliminaires. *Bull. Soc. Path. Exot.* VII. 577.

SWELLENGREBEL, N. H. (1917). Observations on *Blastocystis hominis*. *Parasit.* IX. 451.

TODD, J. L. (1914). The trypanosome of Gambian mice. *Ann. Trop. Med. and Paras.* VIII. 469.

WENYON, C. M. (1910). A new flagellate ("*Macrostoma mesnili*" n. sp.) from the human intestine with some remarks on the supposed cysts of "*Trichomonas*." *Parasitology*, III. 210.

—— (1912). Experiments on the behaviour of Leishmania and allied flagellates in bugs and fleas. *Jl. London School Trop. Med.* II. Part I. 13.

—— (1913). Observations on *Herpetomonas muscae domesticae* and some allied flagellates. With special reference to the structure of their nuclei. *Arch. für Protist.* XXX. 1.

—— (1915). Flagellate forms of *Leishmania donovani* in the tissues of an experimentally infected dog. *Jl. Trop. Med. and Hyg.* XVIII. 218.

WENYON, C. M. and O'CONNOR, F. W. (1917). Human intestinal protozoa in the Near East. Wellcome Bureau of Scientific Research, London.

LEISHMANIA, HERPETOMONAS, AND *CRITHIDIA* IN FLEAS.

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(With Plates XXI and XXII.)

INTRODUCTION.

Most of the observations recorded in this paper would have been published in 1915, if my duties as a doctor and as an officer had not called me to the Army.

Following on my experimental researches (1910–1915), on the transmission of visceral leishmaniasis by means of fleas (*Ctenocephalus canis* and *Pulex irritans*), a very interesting problem arose which still rivets the attention of students of this subject.

In the intestine of various insects and of fleas in particular, certain Protozoa of the herpetomonad and crithidial types have been observed. The morphological, biological and experimental study of these Protozoa in relation to *Leishmania* is particularly interesting.

Patton maintained that this parasite is a true *Herpetomonas*, and called "herpetomoniasis" those infections which we now call "leishmaniasis." Minchin and Wenyon, on the other hand, observed that although *Leishmania* in some stages presents morphological characteristics almost identical with *Herpetomonas* it is distinguished by the fact that *Herpetomonas* is only found in insects, in whose intestines the different species undergo their complete developmental cycle (flagellate and non-flagellate), while *Leishmania* completes its cycle in two hosts, one of which is a vertebrate and the other an invertebrate. In the vertebrate host *Leishmania* produces a disease with characteristic symptoms—"*Leishmaniasis.*"

BIOLOGY AND MORPHOLOGY OF *LEISHMANIA.*

The biological and morphological characteristics of the parasite must be studied, (*a*) in the vertebrate host; (*b*) in artificial cultures; (*c*) in the transmitting host.

(*a*) *Leishmania in the vertebrate host* is non-flagellate, it appears either oval (Plate XXI, fig. 1), or round, with a diameter varying from 2 to 4μ; it may also be pear-shaped (Plate XXI, fig. 3) when it may attain a length of 7μ.

In the parasite we can distinguish two masses of chromatin, the larger is called the nucleus, or trophonucleus, the smaller is called the blepharoplast, or kinetonucleus. Novy has also described a small well defined organ, the rhizoblast. The plasma appears finely granulated and in the large forms shows vacuoles.

The nucleus is rounded and often appears as a compact mass (Plate XXI, fig. 1); occasionally it seems to consist of small grains of chromatin; at times it shows a minute body more deeply coloured, placed centrally or eccentrically, which recalls the karyosome (in *sensu lato*); very rarely the nucleus is absent.

The blepharoplast is rod-shaped (Plate XXI, fig. 1) or pointed (Plate XXI, fig. 2), almost always detached from, but at times attached to the nucleus (Plate XXI, fig. 2). Visentini, who has carefully studied the morphology of *Leishmania* in the vertebrate host, thinks that this attachment of the blepharoplast to the nucleus indicates the nuclear origin of the blepharoplast; sometimes the blepharoplast may be absent (Plate XXI, figs. 4, 5).

In parasites derived from haematopoietic organs, the rhizoblast occurs as a minute body or rod placed between the kinetonucleus and the peripheral zone of the parasite. In canine leishmaniasis, smears from such organs reveal the presence of granular forms which I believe to be connected with *Leishmania*, but I cannot say definitely whether they represent a phase of the developmental cycle and are comparable with the granular forms of trypanosomes, or whether they represent degenerative forms of the parasite.

Reproduction. According to all authorities, *Leishmania* reproduces itself by simple longitudinal division; division of the nucleus and of the blepharoplast is first observed (Plate XXI, figs. 5, 6); then the plasma divides. This form of agamic reproduction leads to the formation of characteristic bodies consisting of several parasites enclosed in a common protoplasm; Ross suspected a sporulation phase; Nicolle on the other hand, considered them to be parasites enclosed in the plasma of the leucocytes whose nucleus might have become detached in the process of preparing the smear.

Besides the reproduction by simple longitudinal division, from which two individuals morphologically indistinguishable are produced (equal division), I have found that sometimes the longitudinal division gives origin to two individuals which can be morphologically distinguished (unequal division); I call the equal division "homomorphous" and the unequal division "heteromorphous."

In heteromorphous division the blepharoplast takes no part whatever in the process of division, which occurs only in the nucleus (Plate XXI, fig. 7).

Visentini described, without giving any explanation, the existence of parasites consisting of plasma enclosing a nucleus but no blepharoplast (Plate XXI, fig. 4). I have decided that such forms result from an unequal division wherein no division of the blepharoplast occurs, the nucleus dividing into two equal similar nuclei, the plasma also dividing immediately. Two parasites are thus

produced, one of which presents a nucleus in which the karyosome is not visible, but which contains the maternal blepharoplast, while the other parasite contains no blepharoplast but shows a nucleus with a karyosome (Plate XXI, fig. 7). This explains the appearance already minutely described by Visentini, and leads us to believe in the nuclear origin of the blepharoplast.

The heteromorphous division above described is probably of significance in the life cycle of *Leishmania*. The development of a parasite which at first is apparently without a blepharoplast is an important phenomenon; I think that some forms of the parasite may represent resistant forms which are of use in the maintenance of the species and which are produced under special conditions. I base this deduction on the fact that I have most frequently met with these forms in the haematopoietic organs of dogs naturally infected with leishmaniasis which was running a chronic course.

(*b*) *Leishmania in artificial cultures.* Rogers, who was the first to cultivate *Leishmania donovani* did so by adding citric acid or citrate of soda in the proportion of 10 per cent. to the spleen or liver juice of individuals affected with leishmaniasis (Indian virus); his experiments being confirmed by Leishman and Statham whilst Longo subsequently cultivated the Mediterranean *Leishmania*. Nicolle, in 1907, cultivated the parasite in the Novy-McNeal medium, and, in 1908, in the Novy-McNeal-Nicolle medium.

Row, in 1912, obtained the cultural development of *Leishmania donovani* (Indian virus) in a haemoglobinised saline solution and Visentini used this medium for cultivating *Leishmania infantum*. *Leishmania* also develops in Bordet and Gengou's medium, in human blood agar and in Jemma's ascitic-agar medium. The temperature is of the highest importance in the cultural development of these parasites; they develop at a temperature of between 18° C. and 25° C., with the best results at 20° C. to 22° C.

After inoculation, from four to five days, on an average, are required before flagellate forms of *Leishmania* appear in the culture fluid. This development may however be completed in a shorter period. Visentini and I once saw a few flagellates appear 48 hours after cultures on N.N.N. medium had been inoculated with the splenic juice of a dog intensely infected with leishmaniasis. After several days non-flagellated forms reappeared and we regard these post-flagellate forms as representing resistant stages.

During the first hours after inoculation the young non-flagellate parasites resemble the forms seen in the blood (peripheral and from haemopoietic organs); gradually they become pear-shaped (Plate XXI, fig. 8) and the flagellum develops (Plate XXI, fig. 9). Such flagellate piriform parasites usually measure 8–12μ in length by 2–3μ in breadth. Besides the pear-shaped parasites there occur some elongated forms (Plate XXI, figs. 10, 11, 12), with a pointed front end from which the flagellum projects. *Leishmania* in cultures reproduces itself by simple longitudinal division and this leads to the formation of slender elongated parasites (Plate XXI, figs. 13, 14, 15).

After some days, there appear flagellate parasites of large size which may

measure up to $26\mu \times 5\mu$. The appearance of these large forms is evidently due to the artificial conditions prevailing in the culture, and, since many of them show vacuolated plasma, they may well represent degenerative forms.

In addition, elongated and spherical forms of rather large size and with granules scattered in the plasma are to be noted. These forms have been regarded as sexual forms; or (Rogers, Marzinowski) as conjugation forms. I have made repeated and prolonged observations in this regard, using a thermostat at 22° C., but I have never observed conjugation to take place.

The morphological study of cultural *Leishmania* both of infantile and canine origin has been carefully followed by Visentini at the Lister Institute. I shall refer to the principal deductions of this writer, dealing chiefly with the morphological identity between the evolutive forms of *Leishmania* in fleas and the forms encountered in cultures.

(*c*) *Leishmania in the transmitting host* (*Ctenocephalus canis*, *Pulex irritans*). Patton, describing the life cycle of the flagellate Protozoa of insects (*Herpetomonas* and *Crithidia*), has distinguished three stages—the pre-flagellate, the flagellate, and the post-flagellate. These stages are also to be noted in the cycle of *Leishmania* in fleas. The pre-flagellate stage is found in the flea's mid-gut; the flagellate stage in the hind-gut; and the post-flagellate stage in the rectum and faeces; these three stages correspond morphologically to those of *Leishmania* in cultures.

In the flea's mid-gut, the parasites show the same characteristics which they present in the peripheral blood and in the haematopoietic organs of infected vertebrates; when fixed and coloured they appear rounded, oval, or pear-shaped (Plate XXI, figs. 16–19), they measure on an average $2–4\mu$ in diameter. The protoplasm assumes a delicate pale blue tint; the nucleus of a reddish violet colour is almost always compact, sometimes granular; the blepharoplast is sometimes absent, as has been noted in parasites from the haematopoietic organs; when it is present it may be rounded or rod-shaped and of the same, or a deeper tint than the nucleus.

Leishmania in this pre-flagellate stage divides longitudinally; first the nuclei and then the plasma. In this manner forms are produced indistinguishable morphologically from those in the haematopoietic organs (Plate XXI, figs. 20, 21, 22).

In some stained preparations however, I have seen certain parasites (Plate XXI, fig. 23), which show two nuclei and a blepharoplast. I have carried out numerous experiments to ascertain whether these might represent sexual forms, but with no success.

In fleas experimentally infected and kept at a temperature of 22° C., after 36 hours from infection I have observed flagellated forms in the intestine, these are morphologically identical with the pear-shaped or elongated cultural forms which have been already described.

In fact, these flagellate, pear-shaped parasites in fleas have almost the same measurements and appearance as the cultural forms, being $7–9\mu$ in

length, exclusive of the flagellum, and 2–4 μ in breadth (Plate XXI, figs. 24, 25, 26). The blepharoplast is situated in front of the nucleus, to which it is more or less contiguous (Plate XXI, figs. 24, 26); in parasites which are nearing the post-flagellate stage it is situated laterally to the nucleus (Plate XXI, fig. 25). The flagellum may attain a length of 20–22 μ; it may develop from a basal granule (Plate XXI, figs. 25, 26, 27), or directly from the blepharoplast (Plate XXI, figs. 28, 29).

The elongated parasites (Plate XXI, figs. 27, 28, 29) are also morphologically identical with the cultural forms; they reach a size of 9–11 μ in length by about 2 μ in breadth; their anterior extremity is pointed; the posterior end is conical, as in the pear-shaped forms. Thus, as in the cultural forms, the various "organellae" may have different shapes and positions in different flagellate individuals.

Leishmania in the flagellate stage reproduces itself in fleas by longitudinal division (Plate XXI, fig. 28); this takes place in the same way as that described for the cultural forms. Either the flagellum, or the blepharoplast, or the nucleus may divide first; the plasma divides last. Two equal parasites are thus produced, and I have never met with unequal division.

In my numerous researches with naturally infected fleas I have very rarely found this flagellate stage; it has seemed to me that this stage may have some connection with the nutrition of the flea; I have more easily found it in fleas whose mid-gut still contained some undigested blood. This observation is of considerable value; if it should be confirmed it will provide a distinctive character between the developmental forms of *Leishmania* in fleas and the common *Herpetomonas* and *Crithidia* of insects, because these latter constantly show all stages, both flagellate and non-flagellate.

The flagellated *Leishmania*, after more or less active multiplication in the intestine of the flea, undergoes certain modifications in structure; the blepharoplast first approaches the nucleus, in some cases coming to lie beside it, the basal granule follows and, in this stage, the flagellum is attached along the anterior third of the body (Plate XXI, fig. 25). In successive stages the anterior, rounded extremity of the parasite becomes pointed and a very short flagellum projects from it; the posterior end, which was pointed, almost conical, becomes rounded and the parasite assumes a pear-shaped form (Plate XXI, fig. 30); later, the flagellum entirely disappears (Plate XXI, figs. 31, 32), the plasma appears of a red colour, the nucleus, the blepharoplast and the basal granule of a deep red colour; still later, the basal granule disappears, the blepharoplast becomes absorbed in the nucleus (Plate XXI, fig. 33); finally, the nuclear chromatin breaks up into numerous granules distributed in the plasma (post-flagellate stage) (Plate XXI, fig. 34). These post-flagellate bodies become provided with a cystic membrane and then the internal contents stain with difficulty. Cystic forms of flagellate Protozoa have been described; *e.g.*, cysts of *Trypanosoma grayi* in the mid-gut of *Glossina palpalis*, which were discovered and described by Minchin. During encystment I have never ob-

served nuclear multiplication, such as has been described by Wenyon in similar forms of *Herpetomona's muscae-domesticae*.

The post-flagellate forms of *Leishmania*, which are expelled with the faeces, help to keep the species in existence and to spread it; this dispersal takes place through the infection *per os* of other fleas and perhaps also of other insects. My experiments, as also those of Archibald (1914), lead me to believe that these post-flagellate forms are also capable of infecting, by the alimentary tract, dogs and healthy children; this is an example of the contaminative mode of infection.

The description of the developmental cycle of *Leishmania* in my previous notes has been confirmed by Alvarez and Sergent.

Alvarez described the occurrence of *Leishmania*, in the pre-flagellate, flagellate and post-flagellate stages, in the intestine and faeces of fleas from a dog infected experimentally with infantile leishmaniasis; Sergent, in collaboration with Lhéritier and Lemaire, described *Leishmania* from the intestinal contents and faeces of fleas taken from a dog infected naturally with leishmaniasis. The studies of Sergent and his collaborators are highly important, because these writers, repeating my experiments with infected fleas, also succeeded in transmitting leishmaniasis to a healthy dog.

The study of the developmental cycle of *Leishmania* in the flea indicates the possibility that there may be two methods of infection of the vertebrate host; the method of inoculation, by the penetration of the parasite through the skin, and the method of contamination, by the post-flagellate forms *per os*.

This possibility of a double mode of infection explains not only the widespread distribution of leishmaniasis in dogs, but also the domestic localisation of the disease, members of the same family becoming infected by fleas, which themselves infect one another by means of the faeces.

LEISHMANIA, HERPETOMONAS, CRITHIDIA IN FLEAS.

The morphological study of the Protozoa of herpetomonad and crithidial types discovered in fleas reveals characteristics which differentiate them from the *Leishmania* which I have discovered in *Ctenocephalus canis* and *Pulex irritans*.

These distinctive characters are most evident in the flagellate stage; in this stage the dimensions of *Leishmania* in fleas, as given above, are very different from those of the various species of *Herpetomonas* and *Crithidia* which have been discovered up to the present in the *Pulicidae*.

The Flagellates observed by Sangiorgi in fleas (*Pulex serraticeps*) attained a length of 16·6μ with a breadth varying from 3–6μ. The flagellum in these forms is as long as, or longer than the body; *Herpetomonas ctenophthalmi* described by Mackinnon in *Ctenophthalmus agyrtes* also attains a length of 22μ, without including the flagellum, and a breadth of 2μ (Plate XXII, fig. 1); the *Herpetomonas* discovered by Wenyon in *Pulex irritans* is about 30μ long,

without the flagellum (Plate XXI, figs. 37, 38); *Crithidia pulicis* discovered by Porter in *Pulex irritans* may even reach a length of 65μ, including the flagellum (Plate XXII, fig. 12); and *Crithidia hystricopsyllae* discovered by Mackinnon in *Hystricopsylla talpae* is 18μ long, without the undulating membrane and the flagellum (Plate XXII, fig. 8). Forms notably larger than *Leishmania* are found also in *Herpetomonas ctenopsillae* described by Laveran and Franchini.

The anterior end of the young pear-shaped forms of *Leishmania* is rounded and is crossed by the flagellum, while in the Insectan Flagellates it is always pointed and drawn out along the flagellum. The posterior end of these forms in *Leishmania* is pointed and almost conical, while in the insectan flagellates it is rounded. *Leishmania* also sometimes shows the anterior end slightly pointed and the posterior end rounded (Plate XXI, fig. 31), but in this case the parasites are always short and thick, with a very short flagellum, and the blepharoplast is very close, or attached to the nucleus; these individuals represent only a stage leading on to the post-flagellate form.

The elongated forms of *Leishmania* in fleas, which are absolutely identical with the cultural forms, show a pointed anterior end, from which the flagellum projects, and a posterior end, which is abruptly truncated, almost conical. It is to be noted, however, that in the *Herpetomonas* described by Mackinnon in *Ctenophthalmus agyrtes* (Plate XXII, figs. 1–4), and by Wenyon in *Pulex irritans*, the posterior end is drawn out in a filiform prolongation (Plate XXI, figs. 37, 38).

Finally, *Leishmania* is differentiated from *Crithidia* by the complete absence of an undulating membrane (Plate XXII, figs. 8, 12, 13, 14, 15).

Herpetomonas and *Crithidia* of fleas, according to Mackinnon, Nöeller and Fantham, may show large forms, which are rounded or elongated (Plate XXII, figs. 5, 6, 11); in this case the ends are rounded or obtuse, the parasites possess a large nucleus and blepharoplast, and many granules distributed through the vacuolar plasma (Plate XXII, figs. 8, 11); the flagellum in these forms is very short and is continued into the body of the parasite as a conspicuous rhizoblast, more or less deeply coloured. I have never seen similar forms in the developmental cycle of *Leishmania* in fleas.

In studying the relations between *Leptomonas* of *Ctenocephalus canis* and *Leishmania* of human and canine origin, Chatton has recently observed that in cultures of *Leptomonas ctenocephali* large forms appear which he calls "aciculées flagellés," which present a body "rubanné" and "torder." We can thus distinguish at once between the cultural forms of *Leptomonas ctenocephali* and those of *Leishmania*.

Also the *Herpetomonas* discovered by Balfour in *Pulex cleopatrae* has certain stages, the dimensions and morphological characteristics of some stages of *Leishmania* in human and canine fleas; these, however, are differentiated morphologically in other flagellate stages, when the posterior end is filiform, as in the *Herpetomonas* found by Mackinnon in *Ctenophthalmus agyrtes* and by

Wenyon in *Pulex irritans*, while in *Leishmania*, as has been already stated, the posterior end is conical.

It can be deduced from these indications that an accurate study of the complete developmental cycle of the various *Herpetomonas* and *Crithidia* already described in fleas reveals certain morphological differences between these and the developmental forms of *Leishmania* in the same insects; on the other hand, the morphological study, if it be not supplemented by biological and experimental data, may be sometimes insufficient.

In this respect I repeat to-day what I have written ever since I began these studies in 1911, "given the constant presence of Protozoa, often morphologically similar, in the intestine of insects, anyone who studies their developmental cycle rather than their morphology is able, by means of experiments, to infer whether the said Protozoa are etiological agents of disease."

It is, in fact, necessary to bear in mind that the herpetomonad and crithidial forms found in the intestine of fleas are not always Insectan Flagellates, since developmental stages of trypanosomes which possess a herpetomonad or crithidial phase also occur in fleas. Minchin and Thomson found in the rat flea (*Ceratophyllus fasciatus*) the developmental stages of *Trypanosoma lewisi*; this parasite, in some of its forms, assumes all the characteristics of *Herpetomonas*; similar phases of *T. lewisi* have also been discovered in *Ctenocephalus serraticeps* (Nöeller), in *Pulex irritans* (Wenyon), and in *Xenopsylla cheopis*, experimentally infected; we cannot, therefore, exclude the possibility that they may be found in fleas infected in nature.

In reference to the relations between pathogenic forms of *Herpetomonas* and Trypanosomes the researches dealing with *Schizotrypanum cruzi* are interesting; in the digestive tract of *Triatoma megistus* taken from the houses of Minas Geraes, Brazil, Chagas observed Protozoa of the herpetomonad type; monkeys inoculated with the intestinal contents of these *Hemiptera* became infected with Trypanosomes; this discovery led Chagas to ascertain the existence of a trypanosomiasis previously unknown, which from its clinical symptomatology has been called "parasitic thyroiditis"; the parasite, on account of certain characteristics of its developmental cycle, has been called *Schizotrypanum cruzi*.

Results identical with those of Chagas have been obtained by Lafont in the island of Mauritius, and by Carini at San Paulo. Recently, also, Tejera when examining the intestinal contents of *Rhodnius prolixus* in Venezuela found these *Hemiptera* infected with flagellates which, when inoculated into animals, produced a trypanosome-infection identical with that caused by *Schizotrypanum cruzi*. These interesting investigations of Chagas into trypanosomiasis, or parasitic thyroiditis, indicate the importance which the researches of experimental protozoology upon the various *Herpetomonas* and *Crithidia* found naturally in fleas have in relation to leishmaniasis.

Since 1910 I have been seeking to solve the important problem of the pathogenic significance of the Protozoa of fleas taken from children and dogs

suffering from visceral leishmaniasis in the Mediterranean region. I have carried out experiments the results of which I will briefly relate. Fleas infected with parasites which I defined as of "leishmanial type," taken from dogs and children infected with leishmaniasis, were put upon healthy dogs. These parasites produced in healthy dogs an infection with the characteristic symptomatology of leishmaniasis; and characteristic leishmanial forms were found in the haematopoietic organs. Similar parasites in fleas were inoculated intraperitoneally into white mice and produced in these animals an infection which has been determined by microscopical examination of the internal organs to be identical with leishmaniasis of infantile or canine origin.

My investigations have been confirmed by Sergent, Lhéritier and Lémaire (1912).

While I, and subsequently Sergent and his collaborators, have made use of fleas taken from children and dogs infected with leishmaniasis, Sangiorgi (1911) began a series of experimental control investigations with a *Herpetomonas* in *Ctenocephalus serraticeps*, of unknown origin; unfortunately, the dogs of which he made use died a few days after the beginning of the experiment and before Sangiorgi was able to arrive at any conclusion.

A. Porter for a long period of time allowed fleas (*Pulex irritans*) infected with parasites of the *Herpetomonas*-type to suck blood from her arm every day, but she did not note any infection in herself. Nöeller, also, obtained negative results from the intraperitoneal inoculation of dogs with the intestinal contents of *Ctenocephalus serraticeps* infected with *Herpetomonas ctenocephali*; Negri obtained similar negative results from puppies (not published), and Chatton from white mice (*Mus albinus*).

To these negative results we can oppose a series of positive results.

Laveran and Franchini obtained in young rats an infection of the internal organs with parasites of a "leishmanial type," by inoculation, by natural means (puncture of the skin and by the digestive tract), making use of dog-fleas naturally infected with *Herpetomonas ctenocephali*. They obtained similar results also with rat-fleas (*Ceratophyllus fasciatus*) infected with *Herpetomonas pattoni*, with *Melophagus ovinus* infected with *Crithidia melophagia* and with *Anopheles* infected with *Crithidia fasciculata*, with *Sarcophaga hemorroidalis*, *Phlebotomus*, and *Blatta orientalis* infected with protozoa of herpetomonad and crithidial types, and finally by injecting cultures of *Herpetomonas ctenocephali* and of *Crithidia melophagia*.

The same writers prepared an emulsion in physiological saline solution of the internal organs of those rats experimentally infected with herpetomoniasis and injected it into dogs and monkeys, in which animals they obtained the reproduction of an infection not distinguishable from that which is observed after inoculation with *Leishmania*.

Fantham and Porter have experimentally demonstrated that Insectan Flagellates, such as *Herpetomonas jaculum* (Léger), of *Nepa cinerea*, *Herpetomonas ctenocephali* (Fantham), *Herpetomonas stratiomyae*, *Herpetomonas pedi-*

culi, and *Crithidia gerridis*, if inoculated intraperitoneally, or if ingested, can live and multiply in the blood and in the internal organs of young rats and of dogs and produce in these animals an infection indistinguishable from visceral leishmaniasis.

In later experiments Fantham and Porter have also demonstrated that a similar herpetomoniasis can be produced also in cold-blooded animals; they have, in fact, produced an infection with *Herpetomonas jaculum* and *Crithidia gerridis*, both by natural means (the digestive tract) and by experimental means (inoculation) in fishes (*Gasterosteus aculeatus*), frogs, toads, lizards (*Lacerta vivipara*) and in snakes (*Tropidonotus natrix*), parasites being present in the internal organs indistinguishable from those met with in leishmaniasis.

But in addition to these experiments the investigations of natural herpetomoniases in animals are highly interesting.

Dutton and Todd in 1903 observed the natural occurrence of a *Herpetomonas* in the blood of a rat in Gambia; Fantham and Porter in 1909 again discovered this *Herpetomonas* in the blood of rats inoculated with spirochaetosis.

Sergent, Lhéritier and Lémaire in prosecuting their researches in Biskra on *Tarentola mauritanica* established that, in making cultures of the internal organs of *Tarentola* on blood agar, in 15·7 per cent. of the cases forms of *Herpetomonas* morphologically indistinguishable from the cultural forms of *Leishmania* were obtained; on this discovery they based the hypothesis that *Tarentola mauritanica* may be a "reservoir" host of *Leishmania tropica*.

Léger has recently affirmed that 2 per cent. of the saurians of the species *Anolis* show a blood infection of *Leptomonas* (*Leptomonas henrici*) which he believes to be of intestinal origin because the same *Leptomonas* was found in 50 per cent. of the individuals examined.

These facts now known to science show that in mammals, as in fishes, reptiles and amphibia (especially in those which are insectivorous) natural herpetomoniases exist, or an infection with an insectan *Herpetomonas* may be induced, either by way of the digestive tract or by inoculation. Herpetomoniasis appears to be an infection which until to-day has not been distinguishable, either by the morphological and biological characteristics of the parasite or by its symptoms, from visceral leishmaniasis.

In my previous work on this subject I have already expressed my belief that the leishmaniases are produced by Protozoa (*Herpetomonas* and perhaps also *Crithidia*) which have adapted themselves to live and multiply in vertebrate hosts; this adaptation is easier in the case of the vertebrate on which the insects are ectoparasitic.

The same species of *Herpetomonas* of insects, under different conditions, may not have any pathogenic action, or may produce pathogenic effects in various degrees in different animal hosts. Referring especially to Mediterranean leishmaniasis this theory explains the acute forms, the chronic forms and the other spontaneously curable forms which have been distinguished in

this disease. I think that in the Mediterranean regions leishmaniasis may be much more frequent than has yet been ascertained, and that slight cases, not easily to be diagnosed, may be particularly frequent.

The facts obtained by my long-continued researches into visceral leishmaniasis in the Mediterranean regions, the epidemiological factors of the close relations between leishmaniasis in children and in dogs, the close relations of contact between children and dogs affected with leishmaniasis and fleas infected with parasitic protozoa which are morphologically and biologically indistinguishable from *Leishmania*, afford ever-increasing confirmation of the results of my investigations, and tend to prove that the visceral leishmaniasis of the Mediterranean is produced by a species of *Herpetomonas* of *Pulex irritans* and *Ctenocephalus canis* which has adapted itself to live in children and dogs, themselves the habitual hosts of these fleas.

BIBLIOGRAPHY.

Alvares, D. (1911). *Med. Contemp.*

Archibald, R. G. (1914). *Journ. Roy. Army Med. Corps.*

Balfour (1906). *Journ. Hygiene.*

Basile, C. (1910–15). *Rend. Acc. Lincei.* Rome.

—— (1913). *Policlinico* (Sezione Pratica).

—— (1912). *Bull. Soc. Pathol. Exotique.*

—— (1915). *Annali d' Igiene.*

Basile, C. and Visentini, A. (1912). *Rend. Acc. Lincei.* Rome.

Chagas, C. (1910, 1911). *Mem. do Inst. Oswaldo Cruz.*

Chatton (1919). *Bull. Soc. Pathol. Exotique.*

Fantham, H. B. (1912). *Brit. Med. Assn.* Liverpool.

—— (1912). *Brit. Med. Journ.*

—— (1913). *Bull. Soc. Pathol. Exotique.*

—— (1915). *Ann. Tropical Med. and Parasitol.*

Fantham, H. B. and Porter, A. (1915). *Proc. Cambridge Philos. Soc.*

—— (1915). *Ann. Tropical Med. and Parasitol.*

Lafont (1912). *Ann. Institut Pasteur.*

Laveran, A. and Franchini, G. (1913–14). *Compt. Rend. Acad. Sci.*

—— (1914, 1915, 1920). *Bull. Soc. Pathol. Exotique.*

Leishman, W. B. (1915). *Journ. Roy. Army Med. Corps.*

Léger (1918). *C. R. S. B.*

Mackinnon, A. (1909). *Parasitology.*

Marzocchi (1911). *Pathologica.*

Minchin, F. A. (1912). *An Introduction to the Study of the Protozoa.*

Minchin, E. A. and Thomson (1915). *Quart. Journ. Microsc. Sci.*

Nicolle, C. (1908–12). *Arch. Inst. Pasteur de Tunis.*

—— (1908). *Compt. Rend. Acad.*

—— (1909). *Ann. Inst. Pasteur.*

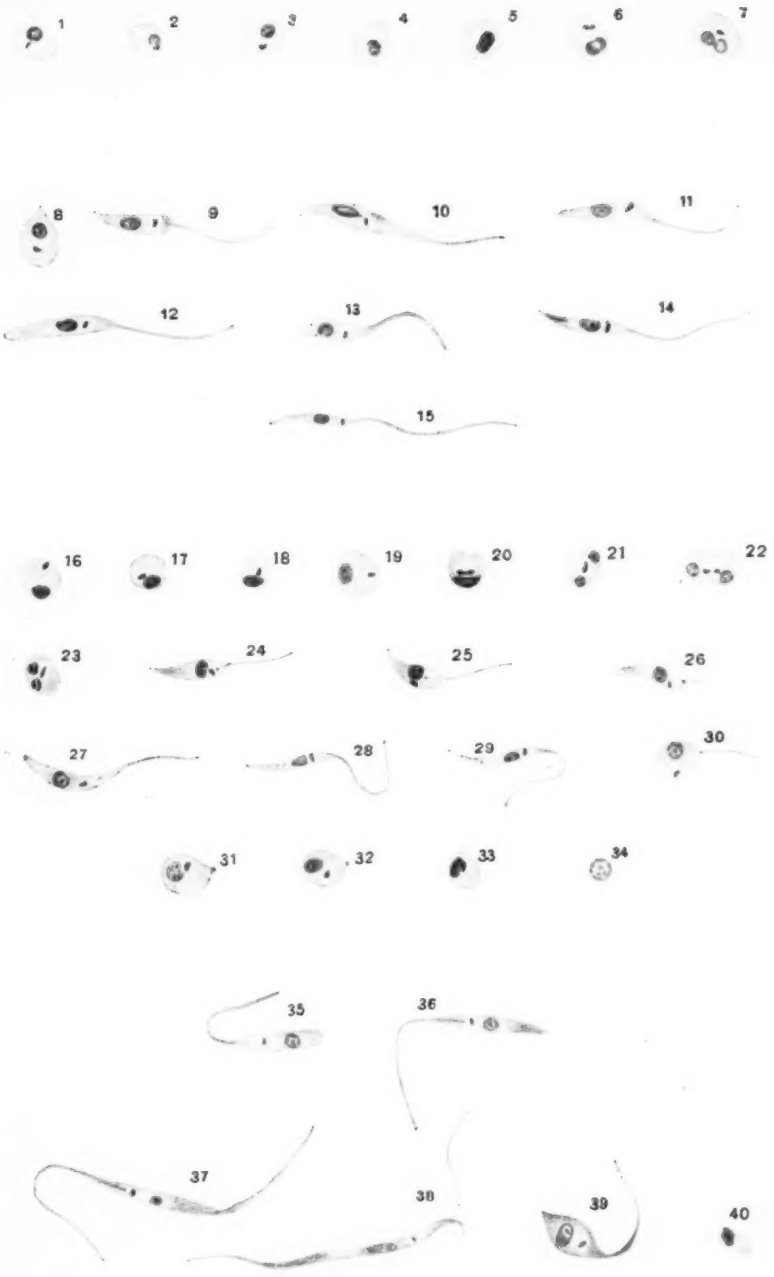
Nöeller W. (1912–14). *Arch. f. Protistenk.*

Patton, W. S. (1915). *Text Book of Medical Entomology.*

—— (1908). *Report of the Bacteriological Section, Royal Institute of Preventive Medicine.* Madras.

—— (1908). *Parasitology.*

—— (1908). *Arch. f. Protistenk.*



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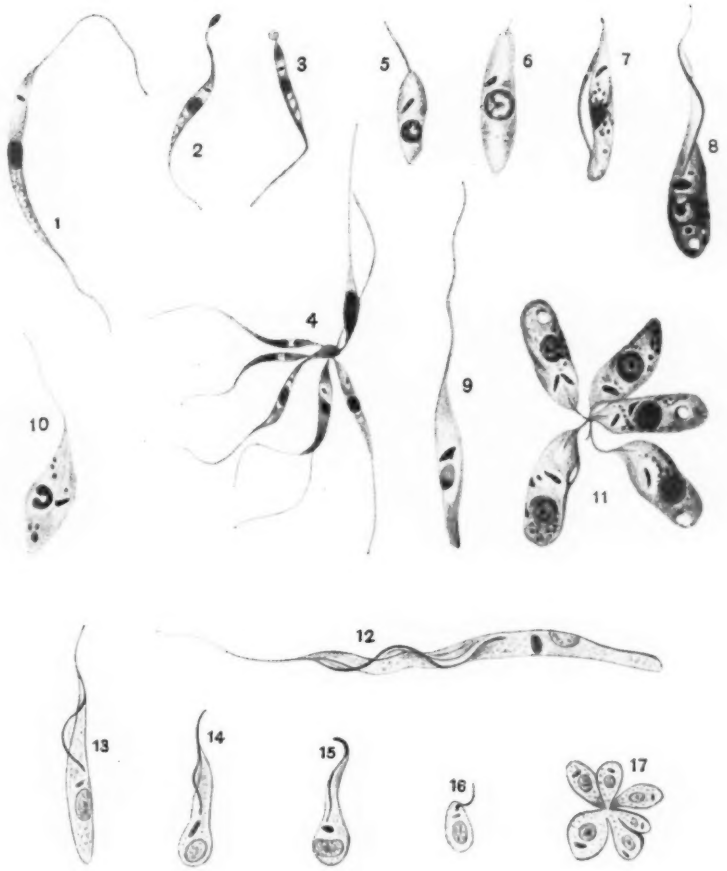
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PATTON, W. S.(1909). *Lancet.*
—— (1909). *Trans. Soc. Tropical Med.*
PATTON, W. S. and STRICKLAND (1908). *Parasitology.*
PORTER, H. (1911). *Parasitology.*
ROGERS, L. (1904). *Quart. Journ. Microsc. Sci.*
ROW, R. (1912). *Brit. Med. Journ.*
SANGIORGI, G. (1911). *Pathologica.*
SERGENT, E. (1912). *Bull. Soc. Pathol. Exotique.*
ED. and ET. SERGENT, A. LHÉRITIER and G. LÉMAIRE (1912). *Bull. Soc. Pathol. Exotique.*
TEIERA, E. (1919). *Bull. Soc. Pathol. Exotique.*
VISENTINI, A. (1910). *Studii intorno ad alcune malattie tropicali della Calabria e della Sicilia.* Rome.
WENYON, C. M. (1912–13). *Journ. London School of Tropical Med.*
—— (1910). *Parasitology* III.

EXPLANATION OF PLATES XXI AND XXII.

PLATE XXI.

(*a*) *Leishmania* in the vertebrate host:
Figs. 1–4. Non-flagellate stage.
Figs. 5, 6. Showing homomorphous division.
Fig. 7. Showing heteromorphous division.

(*b*) *Leishmania* in cultures:
Fig. 8. Young non-flagellate parasite.
Fig. 9. Flagellate form derived from non-flagellate parasites
Figs. 13, 14. Parasites commencing equal longitudinal division.
Fig. 15. Form derived from flagellate parasites by longitudinal division.

(*c*) *Leishmania* in *Ctenocephalus canis*:
Figs. 16–19. Pre-flagellate stages.
Figs. 20–22. Pre-flagellate stages showing equal longitudinal division.
Figs. 24–29. Flagellate stage.
Fig. 28. Parasite commencing equal longitudinal division.
Fig. 30. Parasite approaching the post-flagellate stage.
Figs. 31–34. Post-flagellate stage.

(*d*) *Herpetomonas* in *Pulex irritans* (a specimen kindly sent me by Dr Wenyon):
Figs. 35–39. Different stages of flagellate parasites in the flea's intestine.
Fig. 40. Parasite in post-flagellate stage.

PLATE XXII.

Figs. 1–7. *Herpetomonas ctenophthalmi* (after Mackinnon).
Figs. 8–11. *Crithidia hystricopsillae* (after Mackinnon).
Figs. 12–17. *Crithidia pulicis* (after Porter).

ENDOLIMAX KUENENI N.SP., PARASITIC IN THE INTESTINAL TRACT OF THE MONKEY *MACACUS CYNOMOLGUS.*

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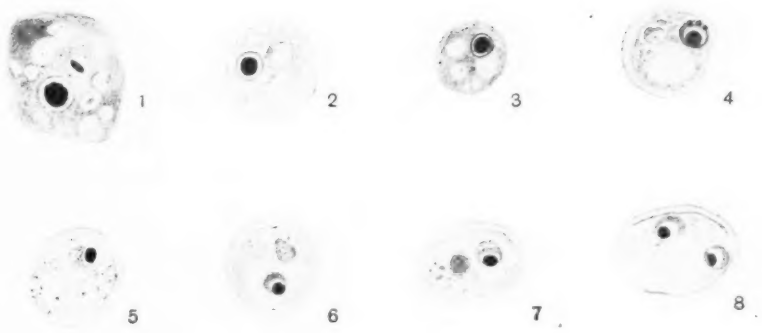
(With Plate XXIII.)

This amoeba was found in the large intestine of a *Macacus cynomolgus*, that was killed because it suffered from paralysis of the hind legs and emaciation. The autopsy showed these symptoms to be dependent on a general tuberculosis, in which, besides many other organs, the spinal matter was involved. Microscopic examination of the intestinal contents revealed the presence of motile amoebae and cysts. Except some small tubercular foci in the serosa no pathological lesions could be detected in the intestine. Close examination of the mucosa failed to reveal any ulceration; blood and mucus were totally absent.

When alive, the motile amoebae did not show a distinct nucleus. The ectoplasm was only visible where pseudopodia were being formed and it was apparently absent in the resting amoeba. The endoplasm was vacuolated, some vacuoles containing small food-particles, mostly bacteria. The amoebae measured 7–12 μ when rounded. The living cysts closely resembled iodine-cysts as described by Wenyon and O'Connor (1917). When the cysts were treated with Weigert's iodine solution, the resemblance with iodine-cysts was emphasised by the appearance of a dark reddish brown stained vacuole measuring $\frac{1}{3}$–$\frac{2}{3}$ of the cyst's diameter. Moreover, just as in iodine-cysts, Weigert's solution failed to produce a clear nuclear picture. The diameter of the cysts varied between 7 and 10 μ; they were round or oval-shaped.

On staining with Delafield's haematoxyline or according to Heidenhain's method as modified by Brug (1919), the amoebae showed the nuclear structure which Kuenen and Swellengrebel (1917) consider to be characteristic for the genus "*Endolimax*" (Plate XXIII, Figs. 1–3). The resemblance of the cysts still held good in stained films. The great majority of the cysts from the monkey were mononuclear, binucleate cysts were very rare (Fig. 8). The nucleus consisted of a darkly staining, large, homogeneous looking, excentrically situated, round caryosome, on one side surrounded by a crescent-shaped, less intensely coloured mass. The latter showed a granular structure more distinctly in



Endolimax kueneni (× 1500).

Fig. 1. Amoeboid form, Delafield's haematoxylin.
Figs. 2, 3. The same, iron haematoxylin.
Fig. 4. Cyst, Delafield's haematoxylin.
Figs. 5–7. Cysts, iron haematoxylin.
Fig. 8. Cyst with two nuclei, iron haematoxylin.

Delafield preparations (Fig. 4) than in Heidenhain-stained films (Fig. 5). Caryosome and crescent were separated by a narrow clearly stained area. The iodophil vacuole appeared as an empty area. The cysts had a double contour, the outer limit of the cyst-wall being visible as a faintly coloured line.

The stained cyst almost always showed a structure which is absent in human iodine cysts. In the protoplasm there occurred a sharply limited area, round or somewhat irregularly shaped, whose periphery stained more darkly than the surrounding protoplasm whilst the centre stained like the protoplasm (Plate XXIII, Figs. 4, 6). In the minority of the cysts this structure stained homogeneously, without a clear centre (Fig. 7).

But for this darkly staining protoplasmic area in the cysts from the monkey's intestine these might be considered identical with the iodine-cysts of human origin. The constant presence of the darkly staining area in the monkey's parasite and its absence in the human parasite, suffices to differentiate these two amoebae. I therefore regard the former as a separate species, for which I propose the name *Endolimax kueneni*.

REFERENCES.

Brug (1919). La coloration des entamibes intestinales des selles. *Bull. Soc. Pathol. Exot.* xii. 71.

Kuenen and Swellengrebel (1917). Korte beschrijving van enkele minder bekende protozoën uit den menschelijken darm. *Gen. Tijdschr. voor Ned.-Indië*, lvii. 496.

Wenyon and O'Connor (1917). The character and diagnosis of the various intestinal protozoa in man in Egypt, etc. *Journ. Roy. Army Med. Corps*, xxviii. 152.

ON SOME DIGENETIC TREMATODES IN JAPAN.

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(With Plates XXIV—XXVI.)

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INTRODUCTION.

THE present paper is the outcome of my examination of a collection of trematodes from various parts of Japan (including Formosa). The species of monostome came from a turtle caught in Singapore. Of the material certain species are left unidentified chiefly owing to the paucity of specimens. It may be mentioned that in the following description the parasites are arranged according to their hosts.

At the outset I must express my thanks to Professors Ijima and Goto, Tokyo Imperial University, and Professors Miyajima and Yatsu, Keio University, for their generosity and suggestions.

I. MAMMALIAN TREMATODES.

(1) **Clonorchis sinensis** (Cobbold).

It is well known that this species infests man, the cat, the dog and the pig. The rabbit, the guinea-pig and the rat can be artificially infested. Although it usually lives in the liver, yet sometimes it is found in the pancreas and even in the duodenum. As to the structure of this species the reader may refer to the papers by several authors, including my own, on its anatomy and life-history[1]. It may here be mentioned that in my former paper the conclusion is reached that there is no difference at all between Looss's[2] *Cl. sinensis* and *Cl. endemicus*.

(2) **Microtrema truncatum** n. g., n. sp. (Pl. XXIV, fig. 1).

Microtrema n. g.

Diagnosis. Opisthorchiidae. Medium sized. Slightly flattened, but relatively thicker than other genera of the family. Taper anteriorly, truncated posteriorly. Cuticula densely spinose. Both suckers relatively small. Ventral and oral sucker widely separated. Intestinal caeca reaching to near posterior end. Excretory vesicle Y-shaped, stem branching into two arms near posterior end of receptaculum seminis; both arms are very long, run anteriorly close to the median line, and reach the exterior at a point near the intestinal bifurcation. Both testes lie symmetrically, slightly anterior to the branching of the excretory vesicle, and near intestinal caeca. No trace of cirrus-pouch. Ovary slightly anterior to level of both testes, lobate. Large receptaculum seminis present. Laurer's canal without opening. Vitellaria consist of several ascinous groups. Uterus largely anterior to ventral sucker, in the space between both intestinal caeca, and not extending posterior to ovary. Genital opening slightly anterior to ventral sucker. Eggs small and containing mature miracidia. Found in the liver of mammals.

[1] Kobayashi, H. (1917). On the life-history and morphology of the liver-distome (*Clonorchis sinensis*). *Mitt. Med. Fachsch. Keijo*, I.

[2] Looss, A. (1907). Some parasites in the Museum of School of Tropical Medicine, Liverpool. *Ann. Trop. Med. and Parasit.* I.

Type species: *M. truncatum* n. sp.

Microtrema truncatum n. sp.

Diagnosis. Size: 12–13 mm. length, 4–5 mm. breadth in the widest part, 1·5–2 mm. thick. Body tapers anteriorly, truncated posteriorly and commonly turned dorsally. Cuticula with densely lying needle-shaped spines. Oral sucker situate at anterior end. Ventral sucker slightly posterior to middle of body, and smaller than the oral. Testes lie symmetrically, or one being often slightly anterior, margin slightly sinuate. Vesicula seminalis short, spindle-shaped. Prostate glands numerous. Ovary with numerous fine lobes. Receptaculum seminis large. Laurer's canal without opening, ending blindly near dorsal body surface. Each vitellarium consists of about 10 ascinous groups. Uterus with numerous irregular loops, some of them barely reaching the intestinal caeca. Eggs brown, small, 0·026–0·03 × 0·013–0·016 mm. Found in the liver of hog.

Externally this species resembles the genus *Pachytrema* Looss, but from its internal structure it may be referred to *Metorchis truncatus* (Rud.) = *Pseudamphistomum truncatum* (Rud.).

Description. This species was found by Koidzumi (1911) in the liver of the pig in Formosa. It is 12–13 mm. long by 5–6 mm. wide in the widest part and 1·5–2 mm. thick in the thickest part.

Body somewhat tongue-shaped. The postero-lateral margins are parallel, the body narrows quickly anteriorly, and posteriorly it is truncated. Ventral surface slightly convex posteriorly. Oral sucker situated slightly ventrally and usually elongated transversely, it measures 0·6 mm. in width. Ventral sucker situated a little posterior to the middle of the body and 7–7·5 mm. from the anterior end; it is round or transversely elongated and measures 0·3–0·4 mm. Genital opening situated on median line and 1 mm. in front of ventral sucker.

The colour of the specimens in alcohol is pale, a few being brown. The uterus and the vitellaria can be seen shimmering through.

The cuticula is 0·02–0·05 mm. thick. It is armed all over with spines except postero-ventrally; the spines are specially numerous on the ventral surface; they are often arranged in threes or in fours; these groups are in turn disposed in transverse or diagonal rows. The spines are lance-shaped, 0·01–0·02 mm. in length by 0·004–0·006 mm. in breadth basally; those on the ventral surface are larger and project while those on the dorsal surface remain in the cuticula.

The three layers of the cutaneous muscles and dorso-ventral parenchyma muscles are all well developed.

The oral sucker is followed by a short prepharynx which leads to the pharynx; the latter measures 0·5 × 0·4 mm.; the oesophagus is short, being slightly shorter than the pharynx and posteriorly it widens and soon bifurcates into the intestinal caeca, which run parallel with the lateral margins of the

body down to the posterior end and terminate at the blind end, being turned slightly mesially.

The excretory pore is situated at the posterior end. The vesicle is Y-shaped, the stem occupying the posterior fifth of the body length and bifurcating near the posterior margin of the receptaculum seminis. The two arms run forward, parallel with each other, along the submedian line against the ventral side of the loops of the uterus, and near the anterior part of the intestinal caeca, they pass over the intestine, where the vesicula becomes continuous with the collecting vessel.

The testes are symmetrically disposed in the hindmost quarter of the body, each lying close to the corresponding intestinal caecum. They are of an irregular, slightly lobate form, with a diameter of about 0·5–0·7 mm. The vasa efferentia arise each from the inner side of the testis, and run anteromedially; at the anterior margin of the ventral sucker they unite into one tube and form the vesicula seminalis. The vesicula seminalis is spindle-shaped, being 0·6 mm. in length. It turns ventrally and is continuous with a short pars prostatica. The prostate glands lie on the ventral side of the vesicula seminalis and around the pars prostatica. The pars prostatica continues directly into the ductus ejaculatorius.

The ovary is situated on the median line, at the same level or slightly anterior to the testes. It is composed of numerous lobes and the whole organ presents a broad triangular shape, with a width of 0·8 mm. The oviduct arises from the dorsal surface of the ovary. The shell-glands are numerous, and lie in the antero-dorsal region of the ovary. The receptaculum seminis is an ellipsoid sac which lies posterior to the ovary; its diameter is 0·3–0·5 mm. The Laurer's canal, after making two or three turns, ends blindly in the dorsal parenchyma. The wall of the distal part of the canal has a peculiar vesicular structure. The vitellaria lie exterior to the intestinal caeca, and extend from the anterior extremities of the intestinal caeca to the level of the receptaculum seminis. Each of the vitellaria is composed of from nine to twelve clearly differentiated ascini. The yolk ducts run in a mesial direction from near the posterior end of the vitellaria and unite at the postero-dorsal margin of the ovary. The convoluted loops of the uterus occupy the entire region circumscribed by intestinal caeca laterally, and the testes and the ovary posteriorly. The vagina runs ventrally, parallel to the ductus ejaculatorius, and opens directly into the latter. The eggs (0·026–0·033 mm. × 0·013–0·016 mm.) are provided with a clearly visible lid; those contained in the distal part of the uterus containing well developed miracidia.

(3) **Eurytrema pancreaticum** (Janson) (Pl. XXIV, fig. 2); and **E. coelomaticum** (Giard and Billet) (Pl. XXIV, fig. 3).

In 1907, Loos[1] described two species of distomes which infest the pancreas of cattle: viz. *Eurytrema pancreaticum* and *E. coelomaticum*. These had been regarded as synonymous. He distinguished them as follows:

1. *E. pancreaticum* is larger and thicker than *E. coelomaticum*; the body-length being more than 10 mm. long and the dorso-ventral thickness 2 mm., while *E. coelomaticum* is not more than 10 mm. in length and 1 mm. thick.

2. The tongue-like appendage at the posterior end of the body is more marked in *E. pancreaticum* than in *E. coelomaticum*.

3. In *E. pancreaticum* the oral sucker is remarkably larger than in *E. coelomaticum* and, in comparison with the body length, the distance between the suckers is greater.

4. *E. pancreaticum* has the distinctly lobate testes, but *E. coelomaticum* less distinct ones.

5. *E. pancreaticum* has larger vitellaria which contain more asci than *E. coelomaticum*. In the former they arise more anteriorly than in the latter.

6. *E. pancreaticum* lays eggs slightly larger than those of *E. coelomaticum*.

I have examined numerous specimens of distomes from the pancreas of *Bos taurus* and have observed these two distinct species as Looss distinguished them. My observations differ from those of Looss in the following respects:

1. The two forms are of about the same size, both measuring between 9·5–11·5 mm. in length. The thickness varies within the same range.

2. The tongue-like appendage on the posterior end seemed to me to have no specific value, individual variation being large.

3. The size and situations of the sucker are good criteria; for in *E. pancreaticum* the oral sucker is 1·8–2 mm. in diameter, while in *E. coelomaticum* it is remarkably small, being 1·3–1·5 mm. in diameter. The ratio of the diameters of both suckers is 10:7 in the former, and 8:7 in the latter.

The distance between the two suckers in the former is 2·2 mm. (measured from the posterior border of the oral sucker to the anterior border of the ventral sucker), while in the latter it is 1·7 mm. on an average.

4. Usually *E. pancreaticum* has distinctly lobate testes, while in *E. coelomaticum* the lobulation is less prominent, but as many intermediate forms exist in both species, this characteristic is of little use.

5. No distinction can be drawn from the relative situations of the vitellaria and the sizes of the eggs.

I distinguish the two species as follows:

E. pancreaticum (Janson). Body leaf-like, 10 mm. long by 4–6 mm. wide. Oral sucker 2·2 mm. in diameter; ventral sucker 1·4 mm. in diameter; the posterior situated at the posterior limit of the anterior half of the body. Testes and ovary usually obscurely lobate, the former consisting of 3–5 lobes,

[1] Looss, A. (1907), *l.c.*

the latter commonly of 3 lobes. Egg 0·045–0·055 mm. × 0·03 mm. Parasitic in the pancreas of *Bos taurus*.

E. coelomaticum (Giard and Billet). Body leaf-like, 10 mm. in length by 4–6 mm. in width, anterior end ordinarily tapering more sharply than in *E. pancreaticum*. Oral sucker 1·3–1·5 mm. in diameter; ventral sucker 1·2–1·4 mm.; ventral sucker situated at the anterior third of the body. Testes and ovary usually indistinctly lobulated. Egg 0·045–0·05 × 0·03 mm. parasitic in the pancreas of *Bos taurus*.

(Several forms were found in association with *E. pancreaticum*; they are distinguished from both the above-described species by their greater translucency and the slender uterus containing fewer eggs. Their real nature remains unknown.)

(4) **Eurytrema satoi** n. sp.[1] (Pl. XXIV, fig. 4).

Diagnosis. Body flat, elliptical, 6–6·5 mm. × 2–3 mm., extremities tapering, posteriorly a tongue-like part is somewhat clearly marked. Oral and ventral suckers about equal in size; ventral sucker situated at the level of the anterior two-fifths. Intestinal caeca extend almost to the posterior end of the body. Testes adjacent to the postero-lateral margin of the ventral sucker. Testes entire, more or less symmetrically disposed. Ovary situated about the middle of the body. Vitellaria small, each consisting of groups of three to four ascini, extending posteriorly from the level of the ovary. Uterus with numerous loops. Egg small, 0·025–0·03 mm. × 0·018 mm. with thick shell and flat lid. Habitat: the pancreas of *Macacus cynomolgus*.

DESCRIPTION. This species infests the pancreatic duct of *Macacus cynomolgus*. The specimens were collected by J. Sato in 1914. Several mature and immature specimens were obtained from a single host.

The body is dorso-ventrally flattened, giving a leaf-like appearance. The form is elongate ovoid or ellipsoid, the greatest width being reached at the posterior third of the body-length. The length is 6–6·5 mm. and the broadest part measures 2–3 mm. in a mature specimen. The lateral margins are gradually convergent anteriorly, while posteriorly they narrow abruptly and at the posterior end a tongue-like appendage is formed. In some cases the broadest part lies at the middle of the body and then the body narrows anteriorly and posteriorly in a like manner, though there is a tongue-like appendage, more or less well defined.

The cuticle is smooth. The oral sucker lies near the anterior end, its diameter is 0·5 mm.–0·55 mm. The ventral sucker lies in the anterior half of the body, and its size is equal, or slightly larger than that of the oral sucker, the diameter being 0·55–0·6 mm.

[1] Since this description was written, I have read the paper by A. Railliet, A. Henry et C. Joyeux (1912), Sur deux Trématodes des Primates, *Bull. Soc. Path. exot.* v. My species resembles *Eurytrema brumpti*, as described by these authors, but differs from the latter in the smaller number of its vitelline glands and in the weaker development of the uterus.

The pharynx lies immediately behind the oral sucker; its dimensions are 0·18 mm. × 0·15 mm. The oesophagus is short, the length being 0·15 mm. The intestinal caeca reach almost to the posterior end of the body and terminate just in front of the tongue-like appendage. The course of the intestinal caeca is slightly sinuate and at the place where the vitellaria are present both caeca are bent inwardly.

The excretory pore opens at the posterior end. The median unpaired part of the vesicle runs anteriorly and at the level posterior to the ovary it divides into arms, which are bent at right angles. After passing over the intestinal caeca they again divide into anterior and posterior branches, which run along the lateral margins of the body and can be traced almost to the ends of the body.

The testes lie immediately postero-lateral to the ventral sucker, their external borders touching or overlapping the intestine. They are round or slightly lobed into 3–5 lobes, their diameter is about 0·4–0·5 mm.; they lie at about the same level.

The cirrus-pouch lies anterior to the ventral sucker and slightly to the right of the median plane. Its size is 0·7 mm. × 0·2 mm. The vesicula seminalis and the pars prostatica make one or two turns. The genital pore opens on the median line, slightly posterior to the bifurcation of the oesophagus.

The ovary lies at about the middle part of the body, and slightly left of the median line. It is slightly elongated transversely, the transverse diameter being 0·18 mm. The vitellaria lie exterior to the intestinal caeca, at the level of the ovary and extending posteriorly for a short distance, and often extend over the intestine. They are composed of three or four ascini. The uterus lies in the space between the two intestinal caeca, often reaching outside the latter. It first runs posteriorly and then anteriorly, passing through the space between the left testis and the ventral sucker and runs anteriorly along the left side of the ventral sucker. At the anterior border of the ventral sucker it turns mesially and after forming a short vagina, opens exteriorly near the male genital pore. Along the whole course the uterus forms numerous lateral loops. The eggs are fairly numerous, their size is 0·025–0·03 mm. × 0·018 mm. The shell is relatively thick and the lid is flat.

Younger specimens measure 1·5 mm. × 0·45 mm.; they have an ellipsoidal shape, the body tapering at both ends. Both the suckers measure 0·2 mm. in diameter. The ventral sucker lies at the middle of the body. The alimentary canal, the excretory vesicle and the immature genital organs can be recognized.

(5) **Dicrocoelium macaci** n. sp. (Pl. XXIV, figs. 5–8).

Diagnosis. Body-shape variable, the broadest part being near either anterior or posterior part; size 4·5–5 mm. × 1–1·5 mm. Cuticle with minute protuberances, densely crowded on the anterior body surface. Ventral sucker slightly larger than the oral. Intestinal caeca terminating at the posterior third of the body, usually unequal in length. Testes lie slightly posterior to

the ventral sucker, lying symmetrically or obliquely, margin irregularly sinuate. Ovary situated slightly posterior to the left testis, rounded or weakly sinuate. Vitellaria extending throughout the middle third of the body-length. Eggs 0·042–0·045 mm. × 0·025 mm. Habitat: the liver of *Macacus speciosus*.

DESCRIPTION. This species was found in the liver of a Japanese monkey, *Macacus speciosus*. Several specimens were collected by M. Miyajima in 1910.

Body lanceolate, 4·5–5 mm. in length by 1–1·5 mm. in width in the broadest part[1].

The greatest width is reached at the anterior third or fourth of the body-length, where the ovary and the testes are situated (Pl. XXIV, figs. 6 and 7). It narrows more abruptly anteriorly than posteriorly. In some specimens, the broadest part is reached at the posterior third of the body-length (like *Dicrocoelium lanceolatum*) (Pl. XXIV, figs. 5 and 8).

The cuticle is provided with numerous fine conical protuberances especially in the anterior part of the body. These become gradually indistinct posteriorly. The oral sucker lies at the anterior end. It has a round form, and a diameter of 0·25 mm. The ventral sucker is situated at the anterior sixth or seventh of the body-length and is slightly larger than the oral, the diameter being 0·32 mm.

The oral sucker is continued directly to the pharynx which is 0·06 mm. in length. The oesophagus is 0·1–0·2 mm. in length and bifurcates into the intestinal caeca midway between the oral and ventral suckers. The intestinal caeca are slender and end at the posterior third of the body-length. They often differ from each other in length.

The excretory pore opens at the posterior end. The unpaired part of the vesicle runs anteriorly and reaches almost as far as the posterior border of the ovary, where it divides into two arms. Both arms turn laterally, almost perpendicularly, and pass exterior to the intestinal caeca. Each of them again divides into anterior and posterior branches, which run along the lateral body margin.

Several gland cells lie on the lateral side of the oesophagus. The glandular ducts run independently forwards and find their opening on the dorsal side of the oral sucker. The contents of these gland-cells and their ducts are eosinophile.

The testes lie directly posterior to the ventral sucker. In certain elongated specimens in which the broadest part is found anteriorly, the right testis lies anterior to the left (Pl. XXIV, fig. 7). In certain other specimens, which have a similar shape to the above, the testes lie side by side (Pl. XXIV, fig. 6). In those specimens, in which the broadest part of the body is situated at the posterior region, both testes lie usually side by side in the same level (Pl. XXIV, figs. 5 and 8). The shape of each testis is lobate or irregularly square, with a diameter of about 0·4 mm.

[1] Measurements from specimens preserved in alcohol, after fixation in sublimate solution under slight pressure.

The cirrus-pouch lies in the region antero-dorsal to the ventral sucker and opens slightly posterior to the place of bifurcation of the intestine. The pouch contains a tubular, winding vesicula seminalis, a short pars prostatica and a long ductus ejaculatorius. The latter may be evaginated exteriorly as the penis.

The ovary lies posterior to the left testis, near the median line. It has an ellipsoid or slightly lobate form, 0·2 mm. in diameter. The oviduct rises at the postero-lateral margin of the ovary. The receptaculum seminis is a small round sac, 0·06 mm. in diameter. Laurer's canal is slender and ends blindly near the dorsal surface. The vitellaria lie exteriorly to the intestine, extending longitudinally in the middle third of the body length. The uterus lies between the intestinal caeca and runs posteriorly. As it approaches the posterior end of the body it turns forwards and passing between the testes, and the ventral sucker, the uterus is continued to the vagina, which opens to the exterior, alongside the male genital pore. Along the course of the uterus numerous smaller loops are met with. The uterus occupies almost the entire space between the ovary and the intestinal caeca.

The eggs are numerous; they measure 0·042–0·045 mm. long by 0·025 mm. wide and each has a relatively thick elliptical shell.

Here it may be desirable to state the probable affinities of this and other species. There are seven genera of mammalian Dicrocoeliinae: *Athesmia*, *Lyperosomum*, *Brodenia*, *Eurytrema*, *Platynosomum*, *Paradistomum*, and *Dicrocoelium*. Of these, three genera (*Athesmia*, *Lyperosomum* and *Brodenia*) may readily be distinguished, one from another, by the following characteristics:

1. In *Athesmia*, the vitellaria are absent on one side.
2. In *Lyperosomum* the body is elongate, and the testes are situated mesially one behind the other.
3. *Brodenia* has a remarkable serration in the middle part of the body.

In the remaining four genera, the differential characters are as follows:

1. In *Eurytrema*, the body is broad and the testes lie side by side at the same level.
2. In *Platynosomum*, the body is lancet-shaped and the widest portion is situated in the middle of the body. Both the testes and the ovary are also found in the widest part. The former are disposed symmetrically.
3. *Dicrocoelium* is lancet-shaped, and the widest portion is situated rather posteriorly. One of the testes lies a little anterior to the other.
4. *Paradistomum* is oval in outline, broadest near the posterior end, and the testes lie side by side.

All the above four genera have a smooth cuticle, excepting *Dicrocoelium concinnum* Braun, whose cuticle is armed with spines. Looss considers this species as belonging to a new genus, because of the spined cuticle.

In some specimens of *Dicrocoelium macaci* the widest portion is at the

level of the testes and ovary, as in *Platynosomum*; in others, however, the widest portion is in the posterior region as in *Dicrocoelium* and *Paradistomum*. The position of the testes varies considerably; in some specimens they lie symmetrically, as in *Platynosomum*, *Eurytrema*, and *Paradistomum*, while in others they lie obliquely. The cuticle of *D. macaci* is not smooth, being covered with fine conical protuberances. Though these protuberances differ from the ordinary spines of Digenea, yet they may be included in the same category. From these characteristics it seems reasonable to establish a new genus for this species. Since the shape of the body and the situation of the testes are not constant in this species, I am rather inclined to believe that the diagnoses of the above-mentioned genera are in need of revision, even to the extent of abolishing certain genera of this group. This species is included only provisionally in the genus *Dicrocoelium*.

(6) **Fasciola hepatica** Linnaeus.

This species is often found in the liver of *Bos taurus*. As a rule few flukes are found in each host.

(7) **Fasciolopsis buski** (Lankester) (Pl. XXV, figs. 2 and 3).

This species is often found in the swine, as well as in the human intestine, in Eastern Asia. In Japan, so far, it has not been found in man. M. Koidzumi has collected two specimens of this species from a Formosan pig (1911). Later K. Nakagawa (1916) kindly sent me several specimens from pigs in Formosa. As a result of my examination, several interesting features, hitherto unrecorded, have been revealed.

The surface of the body is covered with large, closely-set, scale-like spines (Pl. XXV, fig. 3). It may be mentioned that Leiper[1] states that the human species, *Fasciolopsis buski*, alone, is destitute of spines, while all the other species of this genus are spinose. He also remarks that the spines of this genus are easily shed. Goddard[2] recently observed that spines are present in this species.

The body varies remarkably in size, according to the degree of contraction.

The oral sucker has a longitudinally elongated, conical form. The posterior part of the sucker is surrounded by remarkably well-developed annular muscles, Odhner's prepharyngeal sphincter[3]. The prepharyngeal muscles are directly attached to the sucker. There is a short prepharynx, the posterior half of which is surrounded by well-developed annular muscles. These annular muscles are attached posteriorly directly to the pharynx, as the pharyngeal sphincter is, in its turn, attached to the oral sucker. The pharynx has a

[1] Leiper, R. T. (1913). Observations on certain helminths of man. *Trans. Soc. Trop. Med. and Hygiene*, VI.

[2] Goddard, F. W. (1919). *Fasciolopsis buski*, a parasite of man as seen in Shaohing, China. *Journ. Parasitol.* V. No. 4.

[3] Odhner, T. (1902). *Fasciolopsis buski*. *Centralbl. f. Bakt.* etc. XXXI.

spherical shape. The oesophagus is longer than the prepharynx. The uneven-walled intestinal caeca take a winding course but do not branch.

The excretory vesicle (Pl. XXV, figs. 2 and 3) has most remarkable features. The pore opens on the dorsal surface near the posterior end, and is connected to the vesicle by a short canal. The vesicle has a structure similar to that of the Echinostomidae and the Psillostomidae. The median trunk of the vesicle runs straight forwards to the posterior border of the shell-gland, where it divides into two, each branch running along the lateral margin of the shell-gland. At the anterior margin of the gland they unite again into a single tube, which runs anteriorly. At the posterior end of the ventral sucker it is continuous with the lacunae. The lacunae surround all the organs in the interior part—the two suckers, the prepharynx, the pharynx, the oesophagus and the terminal parts of both genital ducts. Four lateral branches arise on each side from the median trunk of the excretory vesicle, two in the region posterior to the shell-gland, one from each ramus alongside the shell-gland, and one anterior to the shell-glands. There is a closely set network of tubules under the cuticle of the entire body surface, which network is continuous with the vesicle, morphologically the former being a part of the vesicle. The network is more abundant ventrally and anteriorly.

The vesicula seminalis is very long and takes a peculiar spiral course with three turns. At the anterior end of the third turn, it has a caecal appendage which runs posteriorly along the wall of the vesicula seminalis proper, and terminates near the posterior end of the latter. The walls of these two parts are formed of epithelial cells. Externally they are surrounded by the long cirrus-pouch. Almost no space exists between the epithelium and the muscle layers of the cirrus-pouch.

Anterior to the vesicula seminalis with its caecal sac, the cirrus-pouch runs directly forwards, passing along the dorsal surface of the ventral sucker. The epithelial cells at this part are elongated, and this portion was taken for the pars prostatica by Odhner[1], but his conclusion may be doubted, since, as Odhner himself observes, it lacks the glandular cells around it. The real pars prostatica lies further forward, in the antero-dorsal region of the ventral sucker, and is delimited from the vesicula seminalis by a constriction. Numerous cells of the prostate glands lie around it. The pars prostatica is continued distally into a relatively long ductus ejaculatorius, which opens to the exterior. The wall of the ductus ejaculatorius is armed with stout spines. The wall of the cirrus-pouch is closely attached to the male duct, contrary to Odhner's figure.

This, with other species of *Fasciolopsis*, has no affinity with the genus *Fasciola*, as has been supposed by previous authors. The following differences are presented by the two genera:

1. *Fasciola* infests the liver, while *Fasciolopsis* is found exclusively in the intestine.

[1] Odhner, *l.c.*

2. Radical differences are manifested in the structure of the cirrus-pouch and the vesicula seminalis.

3. The ventral sucker of *Fasciolopsis* has a peculiar bulging on the posterior wall, while *Fasciola* has none.

4. The oral sucker of this species has a peculiar prepharyngeal sphincter which is wanting in *Fasciola*.

5. In the parenchyma of *Fasciolopsis* numerous well-developed, longitudinal parenchyma muscles are present, while in *Fasciola* they are wanting.

6. *Fasciola* has branched intestines, contrary to *Fasciolopsis*.

7. Remarkable differences are presented in the excretory vesicle of *Fasciolopsis*.

These and other characteristics show that *Fasciolopsis* has affinity with the Echinostomidae, and even may be considered as an aberrant form of the Echinostomidae.

Consequently, the generic and specific diagnosis of this parasite should be thus modified.

Fasciolopsis Lss., emend.

Diagnosis. Echinostomidae. Body large, flat. Cuticle with scale-like spines. Without well-developed, spinose head collar. Oral sucker with peculiar sphincter muscles along its posterior margin. Excretory vesicle with peripheral network. With long cirrus-pouch, which reaches far behind the ventral sucker. Testes lying one behind the other and terminally branched, likewise the ovary. Vesicula seminalis with spiral wall and a peculiar blind sac. Vitellaria well developed. Uterus relatively long, with several loops. Parasitic in the intestine of mammals.

Type species: *F. buski* (Lank.).

Fasciolopsis buski (Lank.), emend.

Diagnosis. Size 24–27 mm. × 5·5–12 mm. Cuticle with spines, more numerous on the ventral body surface. Oral sucker small. Ventral sucker much larger than the oral, with peculiar posterior cup-like evagination. Longitudinal parenchyma muscles remarkably well developed. Intestinal caeca take a somewhat winding course. Excretory vesicle with a median stem and numerous lateral branches, subcutaneous network, and a large lacuna between the two suckers. Cirrus-pouch is canal-like and extends about one-fourth of the body-length. The greater part of the vesicula seminalis lies posterior to the ventral sucker. Cirrus with fine spines projecting in the lumen. Testes lie in the posterior half of the body. Ovary lies directly in front of the anterior testes, slightly right to the median line. Vitellaria extend from the ventral sucker to the posterior end of the body. Eggs large, elliptical with thin shell, 0·12–0·13 mm. × 0·07–0·08 mm. Parasitic in the intestine of man and pig.

Recently Rodenwaldt[1] has described a new species, *Fasciolopsis fuelleborni*. I have reason to believe that this is *F. buski*. *F. fuelleborni* is distinguished from Odhner's *F. buski* principally by the absence of the caecal sac of the vesicula seminalis. He may have mistaken the caecal sac for the cirrus-pouch. Should his observations be correct, *F. fuelleborni* would rather belong to some genus other than *Fasciolopsis*. *F. rathouisi* Poirier[2], and *F. goddardi* Ward[3] may not be good species.

(8) **Paragonimus westermani** (Kerbert).

This species is parasitic in the lung or the viscera of man, the dog, the cat, the tiger and the pig. I have dealt with the structure and the life-history of this species in other communications[4].

(9) **Fischoederius elongatus** (Poirier), and **Paramphistomum** sp.

These two species are found very frequently and numerously in the first and second stomachs of *Bos taurus*. It is certain that there are several species of Paramphistomidae in Japanese cattle; they need further investigation.

(10) **Watsonius macaci** n. sp.[5]

Diagnosis. Size 11·3 mm. × 6·5 mm. Oral sucker small, 0·75 mm. in length. Posterior sucker (acetabulum) very large, 2 mm. in diameter. In the caecum of *Macacus cynomolgus*.

Description. The following description is based on some ten specimens collected from the caecum of *Macacus cynomolgus*.

Body flattened; broadest part at the level of the posterior fifth of the body; posterior end rounded; anteriorly the body narrows, the narrowing becoming abrupt at the anterior sixth of the body-length. Dorsal and ventral surfaces are slightly convex. Specimens fixed by heat are 11·3 mm. in length, 6·5 mm. in breadth, and 2 mm. in thickness; specimens fixed with sublimate are 2–3 mm. shorter than those killed by heat. Posterior sucker lies at posterior extremity of body; round in contour, margin elevated; diameter 2 mm. Oral sucker lies at the anterior body extremity; its anterior half cylindrical, its cross section being ring-like; posteriorly it flattens and shows a crescent shape in transverse section; its size is 0·015 mm. × 0·45 mm. Postero-laterally a pair of spherical suctorial pouches are present, 0·4–0·5 mm. in diameter.

[1] Rodenwaldt, E. (1909). *Fasciolopsis fülleborni* n. sp. *Centralbl. f. Bakteriol.*, etc. I.

[2] Poirier, S. (1887). Note sur une nouvelle espèce de Distome, parasite de l'homme, le *Distomum Rathouisi*. *Arch. zool. expér. et gen.* (2), V.

[3] Ward, H. B. (1909). *Fasciolopsis buskii, F. rathouisi* and related species in China. *Stud. Zool. lab. Univ. Nebraska*, No. 94.

[4] Kobayashi, H. (1918). Studies on the lung-fluke in Korea. 1: On the life-history and morphology of the lung-fluke. *Mitt. Med. Hochsch.* II. (1919), 2: Structure of the adult worm. *Ibid.* IV.

[5] It is very probable that this species is identical with *Watsonius watsoni* collected by Joyeux from *Cercophithecus callitrichus* (A. Railliet, etc. *Bull. Soc. Path. exot.* V.).

Oral sucker continues into the oesophagus at the posterior median point of the former. Oesophagus 1·6 mm. in length; its lumen is narrow and posteriorly it becomes wider and its posterior fourth forms the pharyngeal bulb. The pharyngeal bulb has a diameter of 0·45 mm. and it directly divides into the caeca. Intestinal caeca broad, running directly and somewhat close to the submedian line and terminating posteriorly immediately behind the posterior testes, *i.e.* at the level of posterior two-fifths of the body-length.

The excretory vesicle lies between the anterior margin of the posterior sucker and the shell-gland; posteriorly it becomes a narrow canal which opens at the point 1 mm. from the posterior extremity. The whole organ lies posterior to Laurer's canal. Two vessels arise from the vesicle and run anteriorly along the inner side of the intestinal caeca.

The genital pore opens in a slight prominence on the median line, slightly posterior to the oral sucker.

One testis lies behind the other at about the middle of the body in the space between the intestinal caeca, partly overlapping the latter. The testes are somewhat separated one from the other. Each testis has four or five lobes; when four-lobed it is cross-shaped, and inclined at an angle of 45° to the longitudinal axis. Each testis is about 1·5–1·8 mm. in diameter. The vesicula seminalis is a broad canal, lying along the median line between the anterior testis and the pharyngeal bulb; it has many lateral loops. At the anterior part of the pharyngeal bulb it continues into a relatively thick *pars muscularis* which runs directly toward the anterior end and soon narrows, forming the *pars prostatica*. The pars prostatica continues directly into the *ductus ejaculatorius*, which unites with the vagina and thus a very short *ductus hermaphroditicus* is formed. Around the ductus ejaculatorius, the vagina and the ductus hermaphroditicus certain muscles are present which represent a primitive cirrus-pouch.

The ovary lies slightly in front of, and to the right of the posterior sucker. Its contour is round or ellipsoidal; its diameter is 0·5 mm. The shell-glands are attached between the point of bifurcation of the intestinal caeca and the anterior margin of the posterior sucker. They lie exterior to the intestinal caeca, partly overlapping them. Each consists of from 8 to 12 groups. The uterus forms several loops between the intestinal caeca and opens into the vagina at the level of the ductus ejaculatorius. The egg is ellipsoid; its size is 0·12 mm. × 0·06 mm. The shell is not thick, and is operculated.

II. AVIAN TREMATODES.

(11) **Prosthogonimus japonicus** Braun.

One specimen was collected by O. Takagi, in a hen's egg, at Ibaraki Prefecture, 1915. Body 4 mm. in length by 2 mm. in width.

III. REPTILIAN AND AMPHIBIAN TREMATODES.

(12) **Polyangium miyajimai** n. sp. (Pl. XXV, fig. 4).

Diagnosis. Size 10–11 mm. × 2 mm. Oral sucker round, 0·3 mm. in diameter. Intestinal caeca with sinuate wall. No trace of pharyngeal bulb. Eggs 0·07 mm. × 0·04 mm. Habitat: in the intestine of *Chelone midas*.

Description. This species was found infesting the intestine of *Chelone midas* at Singapore in 1914. The parasite was collected by M. Miyajima, M. Koidzumi and R. Takano. The specimens were fixed by heat.

The size of a mature specimen is 10–11 mm. × 2 mm. The lateral margins of the body are almost parallel, but posteriorly the body slightly narrows. The posterior end is pointed while the anterior end is rounded. The cuticle is beset with fine, closely set processes, but they are often insignificant. The oral sucker has a round outline, the diameter being 0·3 mm. The oesophagus is narrow, the length being 2 mm. There is no trace of the muscular thickening in the posterior part of the oesophagus. The intestinal caeca are broad and simple and run straight along the lateral margin of the body and terminate near the posterior end.

The excretory pore lies medianally on the dorsal side near the posterior end of the body. The unpaired part of the vesicle runs directly almost to the posterior part of the ovary.

The testes lie 0·2 mm. apart, one behind the other, in the space between the intestinal caeca, the hindermost being situated 2 mm. from the posterior end of the body. They have a round shape, their diameter is 0·8 mm. and they are directly attached laterally to the intestine, the latter often bending exteriorly at the point of attachment. The vesicula seminalis is long and runs in the median plane, slightly bending laterally. Anteriorly the vesicula seminalis reaches the level of the bifurcation of the oesophagus, where it joins the pars prostatica. The pars prostatica is a straight canal, lying in the median line, near the ventral surface.

The ovary lies directly behind the posterior testis, slightly to the right of the median line. The outline of the ovary is somewhat round; the diameter being 0·3 mm. Posterior to the ovary lies the compact mass of the shell-gland. The vitellaria occupy the posterior three-fifths of the body, lying mostly outside the intestinal caeca; a small part, however, is found inside the intestinal caeca posterior to the ovary. The uterus, with relatively few loops, lies between the intestinal caeca. Anteriorly the uterus runs straight along beneath the pars prostatica and the oesophagus, and opens to the exterior through a short vagina.

The genital opening lies in the ventral median line, 0·5 mm. from the anterior end of the body.

The egg has a size of 0·07 mm. × 0·04 mm., with relatively thick wall and a distinct lid. The mature shell-wall is brown in colour.

(13) **Cricocephalus koidzumii** n. sp. (Pl. XXIV, fig. 9).

Diagnosis. Resembles *Cr. albus*, but differs from it by (1) its smaller size, 3·5–3·8 mm. × 0·8–1 mm., (2) its very sinuate testes, (3) its having no trace of a pharyngeal bulb. Habitat: in the intestine of *Chelone midas*.

DESCRIPTION. This species was found in the stomach of *Chelone midas*, captured at Singapore. The specimens were collected by M. Miyajima and others and were fixed by heat.

The size is 3·5–3·8 mm. × 0·8–1·0 mm. Body somewhat flattened dorso-ventrally; lateral margins ventrally curved except in the cone-shaped part. The posterior end truncated; the broadest part is near the posterior end and the body either slightly narrows anteriorly, or both lateral margins run almost parallel; at the "shoulder" it abruptly narrows and forms a cone-shaped anterior end; protuberant postero-laterally.

The oral sucker lies near the anterior end of the body, its diameter being 0·3 mm. The cuticle of the body surface is smooth. The oesophagus is 0·5 mm. in length. The intestinal caeca reach almost to the posterior end of the body. Each has numerous (20–30) diverticula, which undergo further sub-division.

Excretory pore opening at the posterior end of the body. The unpaired part of the vesicle runs forward, beyond the level of the posterior margin of the testes, and then divides into two.

Both testes lie symmetrically in the posterior part, their distance from the posterior end being 0·4 mm. They lie exterior to the posterior trunk of the intestine. They have a somewhat lobate or sinuate form, 4–5 lobes being present. The width of each testis is about 0·2 mm. The intestinal caeca bend inwards at the place of attachment of the testes. The vesicula seminalis lies in the anterior part of the posterior half of the body; it is a broad canal with numerous irregular lateral bends. The cirrus-pouch is spindle-shaped and continues anteriorly into the ductus ejaculatorius.

The ovary lies slightly anterior to the level of the testes, somewhat to the right of the median line. It has somewhat irregular lobes, and its diameter is 0·15 mm. Postero-medially to the ovary are the shell-glands. The vitellaria are small, lying exteriorly to the intestinal caeca. They extend shortly anterior to the level of the ovary. Each gland consists of about 15 groups. The uterus is surrounded by the intestinal caeca, the ovary, and the posterior end of the ductus ejaculatorius. It has many transverse loops which lie in close contact with one another. The vagina is very well developed, running along to the left of the ductus ejaculatorius. The distal part of the vagina has long spines in its cuticular wall.

The genital opening lies left of the median line 0·7 mm. from the anterior end of the body. A short genital sinus is present.

The egg bears long, whip-like appendages at both poles. The size of the normal egg is 0·02 mm. × 0·01 mm., while the length of each appendage is

about 0·12 mm. The appendages develop with the growth of the egg-shell, young eggs showing small protuberances only.

(14) **Octangium takanoi** n. sp. (Pl. XXV, fig. 5).

Diagnosis. Size 5·5 mm. × 1·2 mm. in largest specimens. Oral sucker somewhat ellipsoidal. Vitellaria extend posteriorly from level of anterior testis. Eggs 0·07–0·075 mm. × 0·045 mm. Habitat: in the intestine of *Chelone midas*.

Description. This species was found in the intestine of *Chelone midas*, captured at Singapore in 1914. The specimens were collected by M. Miyajima and others and fixed by heat.

Body elongate, breadth uniform in main part of the body, slightly narrowing anteriorly and posteriorly. The general appearance resembles that of *Octangium hasta* Looss. The size of the largest specimens is 5·6 mm. × 1·2 mm. The oral sucker is somewhat elongated. The oesophageal lumen is wide and its thickening is very remarkable. The bifurcation of the intestine lies at the anterior third. The intestinal caeca are almost straight. The anterior ends of the vitellaria are situated at the level of the anterior margin of the testes, not attaining the level of the oesophageal bifurcation. The egg is decidedly smaller than in other species of the genus, measuring 0·07–0·075 mm. × 0·045 mm.

(15) **Pneumonoeces** sp.

This species, of which I have examined a few specimens only, was found in the lung of *Rana nigromaculata* collected in Tokyo. The cuticle is armed with spines.

(16) **Loxogenes liberum** Seno.

I found many examples of this species in the duodenum of *Rana nigromaculata* collected in Tokyo, etc.

IV. FISH TREMATODES.

(17) **Leptolecithum eurytremum** n. g., n. sp. (Pl. XXVI, fig. 1).

Leptolecithum n. g.

Diagnosis. Hemiuridae. Body moderately large, flat; widest at posterior third. Both suckers well developed; ventral sucker at anterior third of body length. Glandular stomach well developed; intestinal caeca with several lateral windings and terminating blindly near the posterior end. Stem of the excretory vesicle bifurcates near the middle of the body and both arms extend almost to the pharynx; stem and arms both make several lateral windings. Testes lie symmetrically, immediately posterior to the ventral sucker. Vesicula seminalis a convoluted canal lying anterior to the ventral sucker. Ovary vermicular in form, lying transversely near the posterior end

of the body and forming a large loop. Vitellaria consist of several long branches, which are again subdivided. Laurer's canal present; receptaculum seminis absent. Uterus very long, forming several convolutions between the two intestinal caeca. Eggs fairly large, numerous. Habitat: in the air-bladder of fishes.

Type species: *L. eurytremum* n. sp.

Leptolecithum eurytremum n. sp.

Diagnosis. Size of mature distome 13 mm. × 7 mm. Both suckers large, the ventral sucker being the larger of the two. Intestinal caeca make about five lateral bends. Stem of the excretory vesicle a broad canal at its posterior part, but at the level of the shell-glands it narrows abruptly and forms lateral bends between the intestinal caeca; at the middle of body it bifurcates, both arms make similar bends and finally end near the pharynx. Margin of testes entire. Vitellaria consist of about four to five large branches, which divide several times. Ovary a long tube about 2·3 mm. in length, lying transversely and often contorted. Uterus occupies the space between the intestinal caeca and makes about three large transverse bends, each of which has fine undulations. Cirrus-pouch (?) a large ellipsoidal muscular mass, slightly protruding in the depression between the pharynx and the ventral sucker. Eggs 0·048–0·05 mm. × 0·023–0·025 mm. Habitat: in the air-bladder of *Parasilurus asotus* and other fish.

DESCRIPTION. The mature specimens of this species are found in the air-bladder of *Parasilurus asotus* and *Pseudobagrus auranticus*, more commonly in the former. Several specimens ordinarily occur in one host. In the coelom of the above two species of fish and *Hypomesus olidus* and *Richardsonius hakuensis*, immature specimens are found. These immature specimens are commonly found near the anterior part of the coelom or in the vicinity of the anus. The parasite has been collected from various places in the Okayama Prefecture, Sahara (Chiba Prefecture), Kasumiga-ura (Ibaraki Prefecture) and Lake Biwa.

A mature specimen measures 13 mm. long and 7 mm. wide at the broadest part. The body is compressed dorso-ventrally, broadest at the middle or the posterior third of the body, and tapering anteriorly and posteriorly. It has on the whole a leaf-like shape. The ventral surface is often slightly concave. In the fresh state, it appears flesh pink, showing blackish colour, due to the intestinal contents, along the intestine. The living parasites move on slowly by the contraction and elongation of the body.

The cuticle is smooth and measures 0·04 mm. in thickness. The oral sucker has a diameter of 1·2 mm. and is situated on the antero-ventral end. The ventral sucker is slightly larger than the oral, being 1·4 mm. in diameter and is situated at the anterior third. The pharynx is directly continuous with the oral sucker and has an elongate form, the size being 0·85 mm. × 0·5 mm. In the entire preparation the oesophagus cannot be detected, but in sections a

short oesophagus is recognisable postero-dorsal to the pharynx, which soon bifurcates into two intestinal caeca which turn laterally at right angles and for a certain distance show the same structure as the oesophagus, being lined by cuticle. This part corresponds to the crop of *Distomum ampullaceum* as described by von Buttel-Reepen[1]. Ultimately, each caecum widens into a sac-like portion, the glandular stomach (Drüsenmagen of the German author), which has special epithelium; each cell of which bears long flagella-like appendages, longest in the posterior portion, and measuring 0·06 mm. in length. The epithelium of the glandular stomach is continuous with the ordinary epithelium of the intestine. Both the caeca turn posteriorly and run along the lateral body margins with characteristic windings, some five turns; and then terminate near the posterior end of the body, where each approaches to the median line. The first turn of the intestine occurs near the anterior margin of the ventral sucker; the remaining four turns are situated posterior to the ventral sucker. The first turn is most remarkable and in some cases the caeca almost meet in the median plane.

The excretory pore opens at the posterior end of the body. The vesicle has the form of an exceedingly elongate Y, the stem and the two arms of which bend laterally several times. In young specimens the stem is straight or slightly curved, while in mature specimens it bends at right angles at the level of the ovary to the right and forms a spindle-shaped broad tube, which becomes abruptly narrower and runs laterally and anteriorly, with two or three transverse bends, in between the intestinal caeca. Its anterior end reaches the middle part of the body and divides into two arms at the median plane. Each arm has a similar course to the stem, and after bending once or twice it reaches the level of the testes and passes over the intestinal caeca, then runs anteriorly along the lateral body margin and reaches as far as the pharynx. At this place it continues into the collecting vessels. The winding course is more marked in large and mature specimens, while in younger examples the entire vesicle usually takes a slightly undulating course.

Both the testes lie at the posterior lateral margin of the ventral sucker, immediately mesial to the intestinal caeca. Each of them has a somewhat round shape, and is 0·7 mm. in diameter. From their antero-mesial border the vasa efferentia arise. They run antero-medially and unite at the antero-dorsal margin of the ventral sucker to form the vas deferens. The vas deferens widens slightly to form the tube-like vesicula seminalis. In the mature specimen it bends 3–4 times, while in the younger form it remains straight. Anteriorly the vesicula seminalis enters into a cirrus-pouch-like muscular organ and continues into the ductus ejaculatorius. Both structures make several windings. The distal end of the ductus ejaculatorius is united with the vagina and forms the ductus hermaphroditicus. The cirrus-pouch-like organ is situated midway between the two suckers; it has an elliptical shape and measures

[1] Buttel-Reepen, H. von (1902). Zur Kenntniss der Gruppe des *Distomum clavatum*, etc. *Zool. Jahrb.* Ab. Syst. etc. XVII.

2 mm. × 1·2 mm. At its anterior part a depression is present on the body surface, the genital atrium. A part of the cirrus-pouch-like organ protrudes into the atrium to form the genital papilla, at the apex of which the ductus hermaphroditicus opens.

The ovary lies at the level between the fifth bend of the intestinal caeca, slightly to the left of the median plane. It is tubular, winding irregularly several times; it measures about 2·3 mm. × 0·15 mm. Its median end opens into a broad and short oviduct. Laurer's canal is present and opens dorsally, while the receptaculum seminis is lacking. The vitellaria are situated at the posterior part of the body around the ovary and between the intestinal caeca. The distal end of the glands passes over the intestinal caeca. They have a dendritic form, are finely branched, and, in the mature specimen, the left and right halves are barely distinguishable. The main branches are 5–6 in number; they are united with one another in the anterior median part and distally they divide into finer branches. The yolk duct arises at the anterior median part. It is short and soon forms a yolk reservoir. The shell-gland is diffuse and the glandular cells have long slender ducts. The uterus makes six loops and is situated between the two intestinal caeca; some parts pass over the caeca to the exterior and run at right angles to the longitudinal axis of the body. In each loop are found still smaller undulations, which are more numerous in the posterior portion. Around the uterus are certain glandular cells which have a similar appearance to the shell-glands, except that they are provided with a shorter duct. Distally the uterus runs directly over the dorsal border of the ventral sucker on the left side of the cirrus-pouch-like organ and is continuous with the vagina, which enters the cirrus-pouch-like organ. After making several windings it unites with the ductus ejaculatorius.

The eggs are numerous, the size being 0·048–0·05 mm. × 0·023–0·025 mm. A distinct lid is present.

It is clearly seen that this species has affinities with the Hemiuridae, especially to the group of *Distomum clavatum*.

I have once found a very young specimen of this species in the gills of *Pseudobagrus auranticus*. The shape is ovoidal, the anterior end being rounded, while the posterior end is somewhat tapering. Its size is 0·35 mm. × 0·2 mm.

The broadest part is the posterior third of the body. The oral sucker lies at the antero-ventral end, the diameter being 0·12 mm. The ventral sucker lies slightly posterior to the middle of the body, the diameter being 0·18 mm. Between the suckers the cirrus-pouch-like organ lies, touching the oral sucker anteriorly and the ventral sucker posteriorly. Both testes lie directly postero-ventrally to the ventral sucker; their shape is ellipsoidal, and each measures 0·05 mm. in diameter. The ovary lies slightly posterior to the testes, between which it is transversely elongated in the median plane; its length is 0·03 mm. The vitellaria are seen as an irregular cell-mass, lying posteriorly to the ovary.

It is very interesting to note that in the coelom of the host no mature

specimens are met with. Similar young specimens are often found in the air-bladder. It seems probable, therefore, that the coelom is not a favourable situation for the maturation of this species.

A cercaria form, which seems to belong to this species, I shall reserve for future description.

(18) **Distomum** sp. from *Thynnus thynnus*.

(A species near *Distomum clavatum*.)

This species infests the stomach of *Thynnus thynnus*. One somewhat immature specimen was obtained from a fish in the Tokyo market.

It is roughly cylindrical in shape, the widest part being situated near the posterior end of the body. Anteriorly it has an almost uniform breadth and terminates in a rounded extremity, while posteriorly it tapers rapidly. The size of a moderately contracted specimen is 25 mm. long and 5 mm. wide in the widest part. The oral sucker is situated at the anterior end of the body, the aperture pointing forwards. It is cup-shaped, measuring 1·1 mm. both in length and dorso-ventral diameter. The ventral sucker lies about 4 mm. posterior to the anterior end. It is protruded remarkably and even pedunculated. The shape is a deep cup-form, and the main axis of the sucker runs obliquely. The length of the sucker is 2 mm. and the aperture of the lumen is 1 mm. in longitudinal diameter. In fixed specimens the anterior part of the body is bent dorsally at the level of the ventral sucker.

The cuticle is very thick and unarmed. The pharynx lies directly posterior to the oral sucker. It is spherical, the diameter being 0·7 mm. The oesophagus curves dorsally, its length being about 0·6 mm., and from it two pairs of the intestinal caeca arise, the anterior and the posterior. The anterior ramus is short, ending near the dorsal part of the pharynx; the posterior ramus reaches almost to the posterior end of the body and has numerous mesial and lateral branchings. The lateral branches are further subdivided.

The excretory vesicle opens at the posterior end of the body. Its unpaired part runs forward for about 5 mm. and then divides into two (?) arms. The unpaired part lies between the two intestinal caeca, its wall following an uneven course, alternating with the branches of the intestine. The lateral arms run posteriorly and, when nearing the posterior end of the body, they turn anteriorly and reach the dorsal part of the oral sucker. It seems probable that they run back again posteriorly. In their course numerous slight undulations are found.

The two testes lie in the median line immediately posterior to the ventral sucker, the anterior one overlapping the posterior. Their shape is spheroidal, the diameter being about 0·5–0·6 mm. Anterior to the ventral sucker is the vesicula seminalis. It is a broad tube and runs along the median plane, winding laterally several times. The pars prostatica is a long winding tube which leads to the ductus ejaculatorius. There is no cirrus-pouch. The ductus ejaculatorius opens on the genital papilla which lies in the genital atrium.

The distal part of the ductus ejaculatorius and the distal part of the female duct are surrounded by several longitudinal parenchyma muscles, forming a cirrus-pouch-like organ.

The ovary lies posterior to the posterior testis. It is round and is smaller than the testes, its diameter being 0·35 mm. The well-developed shell-gland is situated posterior to the ovary. Laurer's canal opens dorsally. The receptaculum seminis is lacking. The vitellaria are long and slender, running along the posterior intestinal caeca. They extend from the level of ovary to within 8 mm. of the posterior end of the body. The loops of the uterus lie between the posterior intestinal caeca and extend backwards almost to the anterior end of the unpaired part of the excretory vesicle. There is no distinct vagina, the whole length of the uterine duct being provided with a cuticular wall. No perfectly formed eggs were found in the specimen under my observation.

(19) **Distomum** sp. from *Muraenesox cinereus*.

This species seems to belong to the Accacoeliinae, Hemiuridae, and may, perhaps, be new; but as I have only one damaged specimen I content myself with its description, and leave its species undetermined.

It measures 11 mm. in length and 5 mm. in breadth in the posterior part, and it narrows anteriorly, the anterior end being 2 mm. wide. Its general form is conical, showing a somewhat circular outline in transverse section. The posterior end terminates roundly.

The cuticle shows remarkable closely set transverse foldings. Subcutaneous muscles well developed in the anterior part, but posteriorly they become weaker.

The oral sucker lies subterminally, its length being 0·6 mm. The ventral sucker is situated about 1 mm. behind the oral, and is deeply invaginated. It is 1·2 mm. in its entire length.

The pharynx continues directly into the oral sucker, its length and breadth both measure 0·8 mm. The oesophagus is longer than the pharynx and makes a dorsal bend, and then bifurcates into intestinal caeca, which form several turns and terminate near the posterior end of the body. At the place of the bifurcation the oesophagus has two anterior diverticula, which end blindly at the level slightly anterior to the pharynx. The diverticula seem to be homologous to the anterior ramus of the intestine in species previously described.

The two testes lie slightly obliquely with respect to one another near the posterior border of the ventral sucker. They are slightly and irregularly sinuate; their lengths are respectively about 0·2 mm. and 0·25 mm. The vesicula seminalis lies at the anterior dorsal part of the ventral sucker; its outline is ellipsoidal and near its middle part a septum-like narrowing is present. The pars prostatica is well differentiated, the length being 1 mm. The wall of this part contains several nuclei which bulge into the lumen.

The main portion of the wall is occupied by the terminal part of the prostate

glands which protrude into the lumen and lie closely side by side. This phenomenon—primary epithelial cells being degenerated and secondary wall formed by union of the ducts of the prostate glands—seem to show the processes of formation and the nature of the cuticula of the body surface and other parts in like manner.

The ovary lies near the posterior end of the body. It is 1 mm. in width and has numerous lobes. Dorsally to the ovary the mass of the vitellarium lies; it is somewhat larger than the ovary and is divided into several lobules. Laurer's canal ends in a vesicular cell mass, similar to that of *Lecithochirium* sp. described later. The uterus is a broad canal which first runs near the ventral body surface, and later near the dorsal, with many windings. Near the anterior dorsal part of the ventral sucker it is continued into the vagina which unites with the ductus ejaculatorius and forms a canal-like genital sinus. The genital pore opens near the oral sucker.

The eggs are numerous, spherical in form, and 0·015 mm. in diameter.

The parasite was found in the stomach of *Muraenesox cinereus*, captured near Okayama (1914).

(20) **Lecithochirium** sp. (Pl. XXVI, fig. 2).

This species infests the stomach of *Muraenesox cinereus*. The length of the body proper is about 3·5 mm. and the appendix 2 mm. The breadth is 1 mm., being broadest in the posterior part of the body proper. The ventral sucker lies 1–1·5 mm. from the anterior end. The diameters of the oral and the ventral sucker are 0·4 mm. and 1·6–1·9 mm. respectively. The size of the egg is 0·021 mm. × 0·01 mm.

This species seems to be new to science[1].

(21) **Brachyphallus** sp.

This species infests the stomach of *Scomber japonicus*. The size of the body is 1–1·7 mm. × 0·35 mm. The diameters of the oral sucker and the ventral sucker are about the same, being 0·12 mm. The pharynx has a diameter of 0·065 mm. The egg is 0·024 mm. × 0·012 mm.

(22) **Lecithocladium** sp.

This species infests the stomach of *Scomber japonicus*. The body-length is 6·5–7·5 mm. The oral and the ventral suckers have lengths 0·45–0·5 mm. and

[1] In the description of this and other genera, Juel, H. O. (1889), Beiträge zur Anatomie der Trematodengattung *Apoblema* (Dujard.), *Bih. Till. K. Svenska Vet.-Akad. Handl.* xv. afd. iv. No. 16; Lander (1904), The Anatomy of *Hemiurus crenatus*, etc. (*Bull. Mus. Comp. Zool. Harvard*, xlv.), and Looss (1908), Beiträge zur Systematik der Distomen. Zur Kenntnis der Familie Hemiuridae (*Zool. Jahrb.* Abt. Syst. etc. xxvi), note the presence of the receptaculum seminis, which has a peculiar structure. After my observation this so-called receptaculum seminis is nothing less than the blindly-ending Laurer's canal which is surrounded by a mass of vesicular cells. A diagrammatic figure of this part is given in Pl. XXV, fig. 6. A similar structure is met with in *Distomum* sp. from *Muraenesox cinereus*, *Brachyphallus* sp., *Lecithocladium* sp. and *Didymozoon* sp., according to my observations.

0·14–0·24 mm. respectively. The elongated pharynx measures 0·45–0·625 mm. × 0·12–0·25 mm. There are no cervical papilla-like process or fringes. The size of the egg is 0·016 mm. × 0·008 mm.

This species appears to be new.

Some of the specimens were parasitised by a sporozoan (Haplosporidia?). The parasites infest only the parenchyma cells of the host.

(23) **Didymozoon** sp. (Pl. XXVI, fig. 4).

This species infests the gill of *Scomber japonicus*. The cyst has an ellipsoidal form, measuring 2 mm. × 1·2 mm. Two individuals are present in one cyst. Both are hermaphrodite and have the same structure. The body is composed of two parts, a narrow anterior and the greatly enlarged posterior part. The anterior part has a length of about 0·7 mm. and a breadth of 0·08 mm. The posterior part measures 1–1·3 mm. in length and 0·55 mm. in breadth. At the junction of the two parts, the posterior part protrudes in two lateral lobes which overlap the anterior part. As Odhner[1] has observed, its form is comparable to that of a mammalian stomach, the anterior part corresponding to the oesophagus.

The oral sucker lies at the anterior end of the body, being placed in a depression. It is pyriform, the length and the width at its widest part being 0·035 mm. and 0·02 mm. respectively. The pharynx is spherical, being 0·025 mm. in diameter. The prepharynx is lacking. The oesophagus is relatively slender; it is divided into two intestinal caeca about at the middle of the anterior part. The intestinal caeca extend almost to the posterior end and they widen in their posterior parts.

The testes, the ovary, the vitellarium and the uterus lie in the posterior portion of the body. They are all very long and make numerous loops. The oviduct arises from the middle of the ovary. The vitellarium has two main trunks which each divide at least twice. The vitellarium extends throughout the whole peripheral part of the posterior portion, while the central part is occupied by the uterus. The ovary lies near the base of the anterior part. There is a Laurer canal, which ends blindly, being surrounded by a group of peculiar cells. Near the basal portion of the anterior part, the uterine walls become abruptly thickened to form a vagina, which runs straight forwards and opens at the anterior end of the body, near the mouth. The eggs are very numerous. They are small, being 0·018 mm. × 0·007–0·009 mm.

Two testes lie near the base of the anterior part of the body. Each of them makes a single loop. At the anterior end the vas efferens arises which soon unites with the vesicula seminalis. The vesicula seminalis is a thick tubular organ running anteriorly between the intestinal caeca and later along the oesophagus. There is a short ductus ejaculatorius which opens at the anterior end, uniting with the vagina.

[1] Odhner, T. (1907). Zur Anatomie der Didymozoon, etc. *Sartryck ur Zoologiska Studier illa gnade Professor T. Tullberg.*

This species resembles in external form *Didymozoon lampridis*, but I am uncertain as to its identity on account of the lack of literature.

(24) **Lepodora** sp. (Pl. XXVI, fig. 3).

This species infests the intestine of *Oncorhynchus masou*. The body is elongated and compressed dorso-ventrally. The broadest part of the body lies slightly posterior to the ventral sucker and anteriorly tapers rapidly, while posteriorly it narrows gradually. Dorso-ventrally the thickest part lies at the level slightly posterior to the ventral sucker. The size is 5–6 mm. in length and 1 mm. in the widest breadth and 0·8 mm. in thickness dorso-ventrally.

The oral sucker is situated at the anterior end of the body, and the ventral sucker at the anterior fourth of the body-length. The diameters of the oral and the ventral suckers are 0·28–0·35 mm. and 0·42–0·6 mm. respectively. Both suckers are round in outline. The cuticle is armed with large scale-like spines which are present all over the body.

The oral sucker is succeeded by the prepharynx, the length of which is 0·55 mm. The pharynx is large and well developed, its length being 0·28 mm. The oesophagus is very short and it rapidly bifurcates into the intestinal caeca. The intestinal caeca are broad and straight and end near the posterior end of the body.

The unpaired part of the excretory vesicle is long and straight. It runs along the ventral body wall and is divided into two short arms at the level of the anterior testis.

The two testes are large and elliptical, and lie between the intestinal caeca, one behind the other, at the middle and the posterior third of the body-length respectively, so closely along the intestine as to press the latter somewhat outward. The length of the anterior testis is 0·7 mm. and the posterior one 1 mm. They lie separated from each other by a short interval. A large and curved cirrus-pouch is present. It extends posteriorly beyond the ventral sucker. The genital opening lies directly anterior to the ventral sucker. The space of the posterior half of the cirrus-pouch is filled with the broad and convoluted seminal vesicle. There is a relatively short pars prostatica lying in the middle part of the pouch. The ductus ejaculatorius has an epithelial wall and opens into the genital sinus.

The ovary lies directly anterior to the anterior testis. It is spherical in shape, its size being 0·3 mm. There is no receptaculum seminis. Laurer's canal opens dorsally. The vitellaria lie along the lateral body margins in the posterior part of the body, partly overlapping the intestinal caeca dorsally and ventrally. The uterus is relatively short, forming several transverse loops between the ovary and the ventral sucker. The vagina is not well marked. At the posterior margin of the ventral sucker the uterus is continuous with a somewhat muscular canal, which has an epithelial wall. This canal, or rudimentary vagina, runs anteriorly along the dorsal border of the cirrus-pouch,

and opens into the genital sinus, which connects the genital ducts with the external genital opening. The eggs are not numerous. The size is 0·035-0·037 mm. × 0·018 mm. and the shell is very thin.

This species differs from *Lepodora* Odhner, 1905[1], in certain characteristics, namely (1) that the genital opening lies directly anterior to the ventral sucker in the median plane; (2) that there is a relatively long genital sinus; and (3) that the receptaculum seminis is not present. In general structure, however, it closely resembles *Lepodora*, but certainly it differs from *Lepodora rachiaena*. It seems to be a new species of the Genus *Lepodora*.

(25) **Phyllodistomum folium** (Ofers.).

I found the parasite in the bladder of *Pseudobagrus aurantiacus* collected in Lake Biwa and Kasumigaura.

(26) **Exorchis oviformis** n.g., n. sp. (Pl. XXVI, figs. 5 and 6).

Exorchis n. g.

Diagnosis. Very small. Body oval to spherical or even broader than long. Anteriorly tapers slightly, posterior end somewhat truncated. Cuticle spinose. Ventral sucker lies anterior to the middle of the body and is smaller than the oral. Alimentary canal consists of the short prepharynx, the pharynx, the short oesophagus and the intestinal caeca, which terminate near the posterior end. Excretory vesicle Y-shaped, arms extend to near the point of the intestinal bifurcation. Genital pore lies directly anterior to the ventral sucker. No cirrus-pouch. Testes lie symmetrically, exterior to the intestinal caeca, at the level of the middle of body; margin entire. Vesicula seminalis large, constricted in the middle. Ovary lies slightly posterior to the ventral sucker, nearly on the median line; smaller than the testis and weakly lobate. Broad Laurer's canal present. No receptaculum seminis. Vitellaria in the anterior lateral region of body. Uterus with several loops in the posterior half of the body, partly extending outside the intestinal caeca. Eggs medium sized; shell thin. Habitat: in the intestine of fishes.

Type species: *E. oviformis* n. sp.

Exorchis oviformis n. sp.

Diagnosis. Size 0·2–3 mm. × 0·2–0·28 mm. Shape often spherical. Oral sucker lies at the anterior end, 0·055–0·077 mm. in transverse diameter. Ventral sucker 0·04 mm. in diameter, situated at the level of the anterior third. Two eye-spots are present in the dorso-lateral region of the oral sucker. Other median pigment spots often present. Pharynx 0·03 mm. in breadth. Oesophagus 0·01–0·015 mm. in length. Intestinal caeca end near the posterior body end. Testes round or ellipsoid. Vesicula seminalis very large. Ovary with four or five lobes. Follicles of vitellaria relatively few in number. Laurer's

[1] Odhner, T. (1905). Die Trematoden des arctischen Gebietes. *Fauna Arctica.*

canal broad and terminating blindly (?) near the dorsal surface. Uterus with several irregular loops lying within and without the intestinal caeca, in the posterior half of body. Eggs 0·04 mm. × 0·02 mm. Habitat: in the intestine of *Parasilurus asotus*.

Description. This parasite is found abundantly in the duodenum and the anterior part of the small intestine of *Parasilurus asotus*. The species occurs very commonly in the fish from various parts of Okayama and Tokyo.

Various stages of the species are found in one host. The average size of mature examples is 0·2–0·3 mm. in length and 0·26–0·28 mm. in breadth[1]. Generally the body is slightly broader than long. Both anterior and posterior ends are round, the anterior part often narrowing slightly. It is compressed dorso-ventrally. It appears as a brown dot to the naked eye, the colour being derived from the eggs in the uterus.

The cuticle is relatively thick, measuring 0·003–0·004 mm. In fresh specimens closely-set, fine spines are found in the cuticle, excepting on the posterior part of the body. In sections, however, these spines appear indistinct, being represented as small protuberances on the surface. Several cephalic glands lie in the anterior end of the body near the oral sucker.

The oral sucker is situated at the anterior end, facing ventrally. In moderately extended specimens, the ventral sucker lies at the anterior third of the body and it is smaller than the oral one, the diameters of the oral and the ventral suckers being 0·055–0·077 mm. and 0·04 mm. respectively; the ventral is deeply depressed.

A pair of round, brown eye-spots are present at the anterior dorsal part of the body, laterally to the oral sucker. There is another median pigment spot lying slightly anterior or posterior to the paired eye-spots. In mature specimens they are indistinct and in some cases they are found disintegrating into several pigment granules.

The pharynx is small. Its breadth is 0·03 mm. The intestinal caeca turn first laterally and then posteriorly along the lateral body margin and terminate near the posterior end of the body, where they turn mesially.

The excretory vesicle is V-shaped, opening at the posterior end of the body. The arms are very broad and lie between the intestinal caeca and anteriorly they reach a point immediately posterior to the oesophagus.

Both testes lie near the middle part of the body on the same level, either on the dorsal side of, or lateral to, the intestine as the case may be. The shape is round or elliptical. The vesicula seminalis is very large and lies dorsally to the ventral sucker. It is 0·15 mm. × 0·05 mm. and its dorso-posterior end almost touches the dorsal body wall. Slightly distal to the middle part, the organ is constricted, the constriction dividing the organ into two unequal parts. Antero-ventrally it narrows into a short ductus ejaculatorius which unites with the vagina and forms a tube-like genital sinus. It opens at the anterior border of the ventral sucker. It is destitute of a cirrus-

[1] As measured in a preserved specimen.

pouch, and no distinctly differentiated pars prostatica is present, excepting a few glandular cells lying around the proximal part of the ductus ejaculatorius.

The ovary lies posterior to the ventral sucker. It is smaller than the testes and has 4–5 lobes. The vitellaria occupy the anterior dorsal part of the body and have a dendritic outline. It is doubtful whether Laurer's canal has an external opening or not; the canal is a relatively broad tube which runs dorsally, side by side with the wall of the vesicula seminalis, and seems to end blindly. The uterus lies surrounded by the intestinal caeca. It forms several loops posterior to the ventral sucker, and some of them go exteriorly beyond the testes. The vagina, relatively long and slender, runs across the ventral sucker and ultimately unites with the ductus ejaculatorius. The egg measures 0·04 mm. × 0·02 mm. and has a distinct lid. The shell is brown and relatively thick. In the distal part of the uterus, eggs are found containing embryos in an advanced stage of development.

I have often found encysted distomes in fresh water fishes, which bear a close resemblance to *Exorchis oviformis*, differing only from the latter in the absence of eggs in the uterus of the former. Young specimens of *Exorchis oviformis* are often observed in the intestine of *Parasilurus asotus*. No eggs are present in such specimens, while they have the same structure as the encysted distomes above mentioned. As the host of *Exorchis oviformis* usually feed on smaller fresh-water fishes, it is probable that this encysted distome is the young stage of the above parasite.

This encysted distome is found in various species of fishes; especially in *Pseudorasbora parva*, various species of *Leucogobio*, various species of *Acheilognathus*, *Carassius auratus*, *Richardsonius hakunensis*, various species of *Zacco*, and *Misgrunus anguillicaudatus*.

The encysted distome lies in the muscles, under the scales or in the fins. The cyst is spherical with a somewhat irregular shape, the diameter being 0·1–0·2 mm. The wall of the cyst is hyaline and structureless. Removed from the cyst, the worm (Pl. XXVI, fig. 6) has an ovoid outline, narrowing anteriorly, while posteriorly it terminates roundly. In the cyst, the worm is curved on itself or shortened lengthwise. The skin is closely beset with fine spines, excepting the posterior part of the body. These spines can be detected only in the fresh state. The oral sucker occupies the anterior end and the ventral sucker is situated slightly anterior to the middle of the body-length. The latter is smaller than the former, the diameters being 0·05 mm. and 0·035 mm. respectively. The parenchyma is transparent. In the anterior dorsal part of the body one pair of eye-spots and one median pigment spot are present. The eye-spots are situated symmetrically on either side of the oral sucker, with the median pigment spot between them. The median spot is often excentric, and in some cases it is wanting entirely.

Posterior to the oral sucker is a small pharynx measuring 0·023 mm. × 0·02 mm. and this is continuous posterior with the short oesophagus which divides into intestinal caeca; these turn outwards and backwards along the

lateral body margin and reach almost to the posterior end of the body. The excretory vesicle is V-shaped and lies between the intestinal caeca and opens at the posterior end of the body. The lumen of the vesicle contains refractile excretion granules. At the middle of the body and laterally or dorsally to the intestine are the testes. They lie at the same level. Directly posterior to the ventral sucker is a small cell-mass which represents the ovary.

Exorchis oviformis seem to have a close affinity with *Stegopa globosa* Linton, which was discovered in the intestine of a marine fish by Linton[1]. The description and the illustration of *Stegopa* are very incomplete, so I cannot identify them. I may add that there is little affinity between the two genera *Siphodera* and *Stegopa*, which are comprised in one family, Siphoderidae, by Linton.

(27) **Steringotrema nakazawai** n. sp. (Pl. XXV, fig. 7).

Diagnosis. Body small, 2·3–3 mm. × 2–2·8 mm.; posterior half broadest. Ventral sucker 1 mm. × 1·5 mm. Alimentary canal consists of a spherical pharynx, short oesophagus and narrow intestinal caeca which run along the lateral margins and end near the posterior end of the body, approaching each other at their distal extremities. Excretory vesicle V-shaped, arms reaching almost to the oral sucker. Testes entire, round, 0·3–0·4 mm. in breadth. Ovary with several lobes, transversely elongated, 0·2 mm. in breadth. Vitellaria consist each of from 5 to 6 groups. Loops of uterus run along the posterior and left side of the ventral sucker. Eggs 0·045 mm. × 0·025 mm. Habitat: in the intestine of *Sparus latus*.

Description. This species infests the intestine of *Sparus latus*, and the specimens were collected by K. Nakazawa at Taka-no-Shima, Awa Province, in 1911.

Body measures 2·3–3 mm. × 2–2·8 mm. The shape is somewhat ovoidal, the posterior half being broadest, and anteriorly it gradually tapers, while posteriorly it ends roundly. The ventral surface is ordinarily flat or concave, while the dorsal surface is somewhat convex. In alcohol the specimen is pale blue.

The cuticle is smooth and relatively thick, being 0·005 mm. The oral sucker is situated at the anterior end of the body, and turned towards the ventral surface, the diameter being 0·1–0·3 mm. The ventral sucker is very large, occupying the main part of the posterior broad part of the body, it is broader than long, measuring 1 mm. × 1·5 mm.

The oral sucker is directly connected posteriorly with the pharynx, without the intervention of a prepharynx. The pharynx is 0·2 mm. × 0·2 mm. A short oesophagus is present. Its length is 0·2 mm. The intestinal caeca run along the borders of the ventral sucker, as far as the posterior border of the latter, and end near the median line. The lumen of the intestine is relatively narrow.

Excretory vesicle V-shaped, and opening on the dorsal surface near the

[1] Linton, E. (1910). Helminth fauna of the Dry Tortugas. II. Trematodes. *Publication No.* 133, *Carnegie Institution.*

posterior end of the body. Both arms run along the inner side of the intestinal caeca. Anteriorly they almost reach the posterior lateral border of the oral sucker.

Both the testes lie at the same level between the postero-lateral border of the ventral sucker and the intestinal caeca. They have a round or ellipsoidal shape, being 0·3–0·4 mm. in diameter. There is a well-developed cirrus-pouch, which is situated between the anterior part of the two intestinal caeca and the anterior border of the ventral sucker and slightly to the left of the median plane. It is somewhat round, the diameter being 0·5 mm. In the cirrus-pouch is the vesicula seminalis and the pars prostatica, the latter being divided into two parts by a constriction; the proximal part is narrower and curved, while the distal part is broader and short. The pars prostatica is long and wide and follows a circular course. In the genital sinus and near the opening of the pars prostatica is an invagination, which seems to perform the function of penis. There is no distinctly differentiated ductus ejaculatorius in the distal part of the pars prostatica, for the latter opens directly into the genital sinus. Around the pars prostatica are numerous prostate glands which fill up the entire space of the cirrus-pouch. The ducts of the glands terminate as long cilium-like processes on the wall of the pars prostatica. The processes lie closely together, their length being 0·02–0·06 mm.

The ovary lies on the posterior median side of the right testis, near the posterior margin of the ventral sucker, and a little to the right of the median plane. It has a somewhat lobate form, being broader transversely. It is smaller than the testis, the transverse diameter being 0·2 mm. The oviduct arises from the median border of the ovary. The vitellaria lie exterior to the intestinal caeca extending along the side of the ventral sucker. Each vitellarium consists of 5–6 ascini, each of which has a special yolk duct which unites with the rest to form a main duct. The main yolk duct runs mesially and unites with its partner from the opposite side in the median plane near the posterior margin of the ventral sucker, and forms a common duct which soon widens into a yolk reservoir and then opens into the oviduct. Laurer's canal is present, and takes a winding course in its proximal part, but finally it runs straight and opens to the dorsal surface. The receptaculum seminis is totally wanting. The loops of the uterus lie side by side with the posterior and left-hand border of the ventral sucker. At the side of the cirrus-pouch it is continuous with the vagina. The female genital opening lies close to that of the cirrus-pouch, in the genital sinus. The proximal part of the uterus widens slightly. The spermatozoa fill up this portion.

The genital sinus lies slightly to the left of the median plane, towards the ventral and anterior margin of the cirrus-pouch. It is nothing more than a depression of the body surface, provided with double circular folds.

The size of the egg is 0·045 mm. × 0·025 mm. The shell is relatively thick and the lid is conspicuous. The eggs in the distal part of the uterus contain embryos at a quite advanced stage of development.

The genus *Steringotrema* Odhner, 1911, has a close affinity with *Didymorchis* Linton[1], and they seem to be identical.

I have in my possession several other species of Digenea, but their examination is incomplete; the publication of their descriptions must be postponed.

EXPLANATION OF PLATES XXIV—XXVI.

PLATE XXIV.

Fig. 1. *Microtrema truncatum* n. g., n. sp. ×10.
Fig. 2. *Eurytrema pancreaticum* (Janson). ×5.
Fig. 3. *Eurytrema coelomaticum* (Giard and Billet). ×5.
In Figs. 2 and 3 merely the outline of suckers and gonads are given.
Fig. 4. *Eurytrema satoi* n. sp. ×18.
Fig. 5. *Dicrocoelium macaci* n. sp. ×20.
Figs. 6–8. Ditto, showing various shapes and situations of the body and the gonads. ×10.
Fig. 9. *Cricocephalus koidzumii* n. sp. ×14.

PLATE XXV.

Fig. 1. *Watsonius macaci* n. sp. ×5.
Fig. 2. *Fasciolopsis buski* (Lank.), showing excretory vesicle. ×3.
Fig. 3. Ditto; longitudinal section of the skin and the underlying tissues of the ventral part of body. ×170.
Fig. 4. *Polyangium miyajimai* n. sp. ×10.
Fig. 5. *Octangium takanoi* n. sp. The uterus is not figured. ×10.
Fig. 6. Diagrammatic illustration of the Laurer's canal, the ootype and their vicinity of *Lecithochirium* sp. ×120.
Fig. 7. *Sterigotrema nagazawai* n. sp.

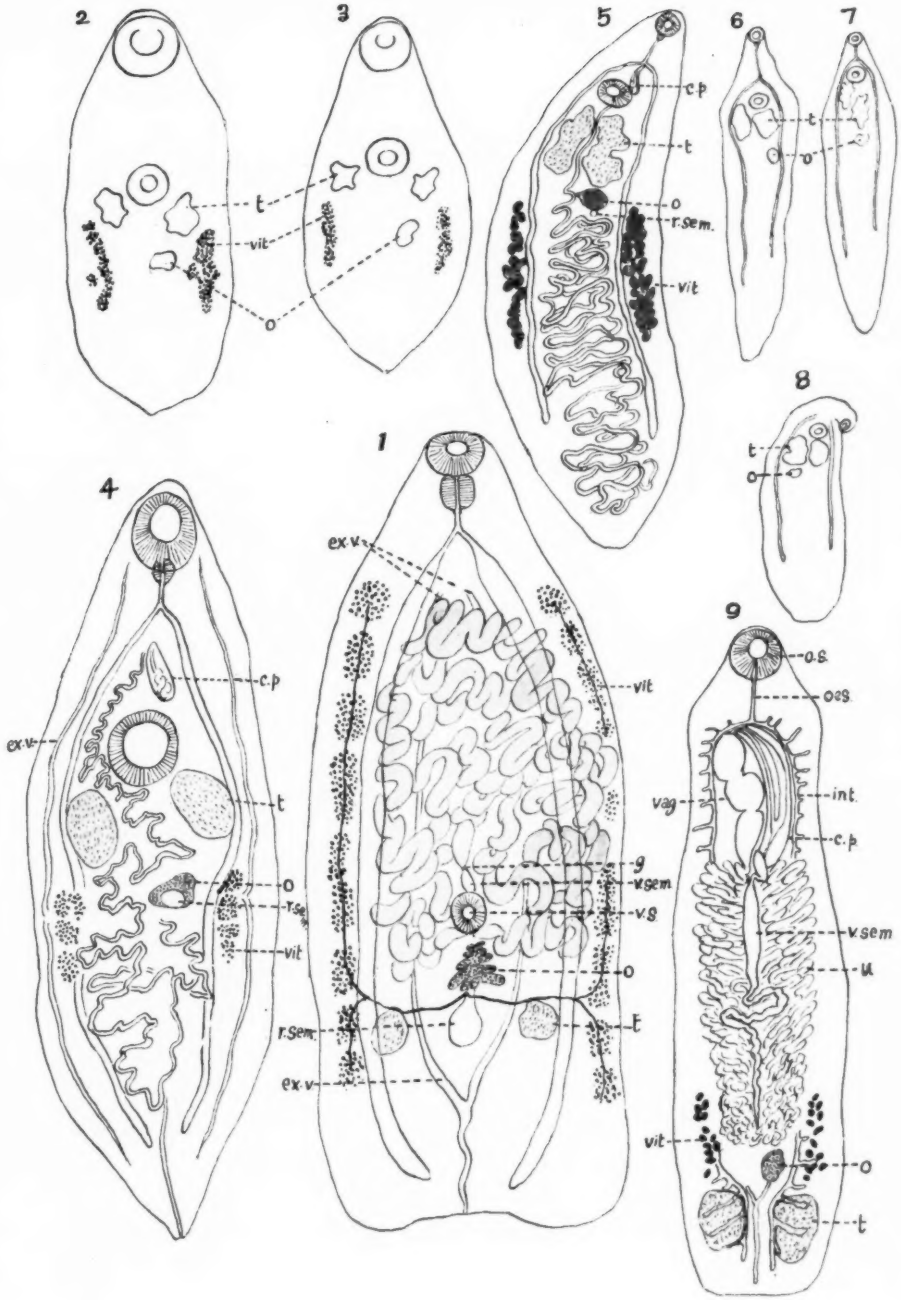
PLATE XXVI.

Fig. 1. *Leptolecithum curytremum* n. g., n. sp. ×10.
Fig. 2. *Lecithochirium* sp. ×10.
Fig. 3. *Lepodora* sp. ×20.
Fig. 4. *Didymozoon* sp. ×40.
Fig. 5. *Exorchis oviformis* n. g., n. sp. ×200.
Fig. 6. Ditto; young, encysted worm. ×40.

REFERENCE LETTERS.

c.p. = cirrus-pouch; *c'p'* = cirrus-pouch-like organ; *cu.* = cuticula; *d. ej.* = ductus ejaculatorius; *ex. n.* = peripheral network of the excretory vesicle; *ex. v.* = excretory vesicle; *g.* = genital pore; *int.* = intestine; *L. c.* = Laurer's canal; *o.* = ovary; *o. s.* = oral sucker; *oes.* = oesophagus; *ot.* = ootype; *ovd.* = oviduct; *p. b.* = pharyngeal bulb, or muscular part of the oesophagus; *p. s.* = posterior sucker; *ph.* = pharynx; *prep.* = prepharynx; *r. sem.* = receptaculum seminis; *s. c.* = subcuticular cells; *sh. gl.* = shell gland; *sp.* = spine; *t.* = testis; *u.* = uterus; *v. sem.* = vesicula seminalis; *vag.* = vagina; *vit.* = vitellarium.

[1] Linton, E. (1910), *l.c.*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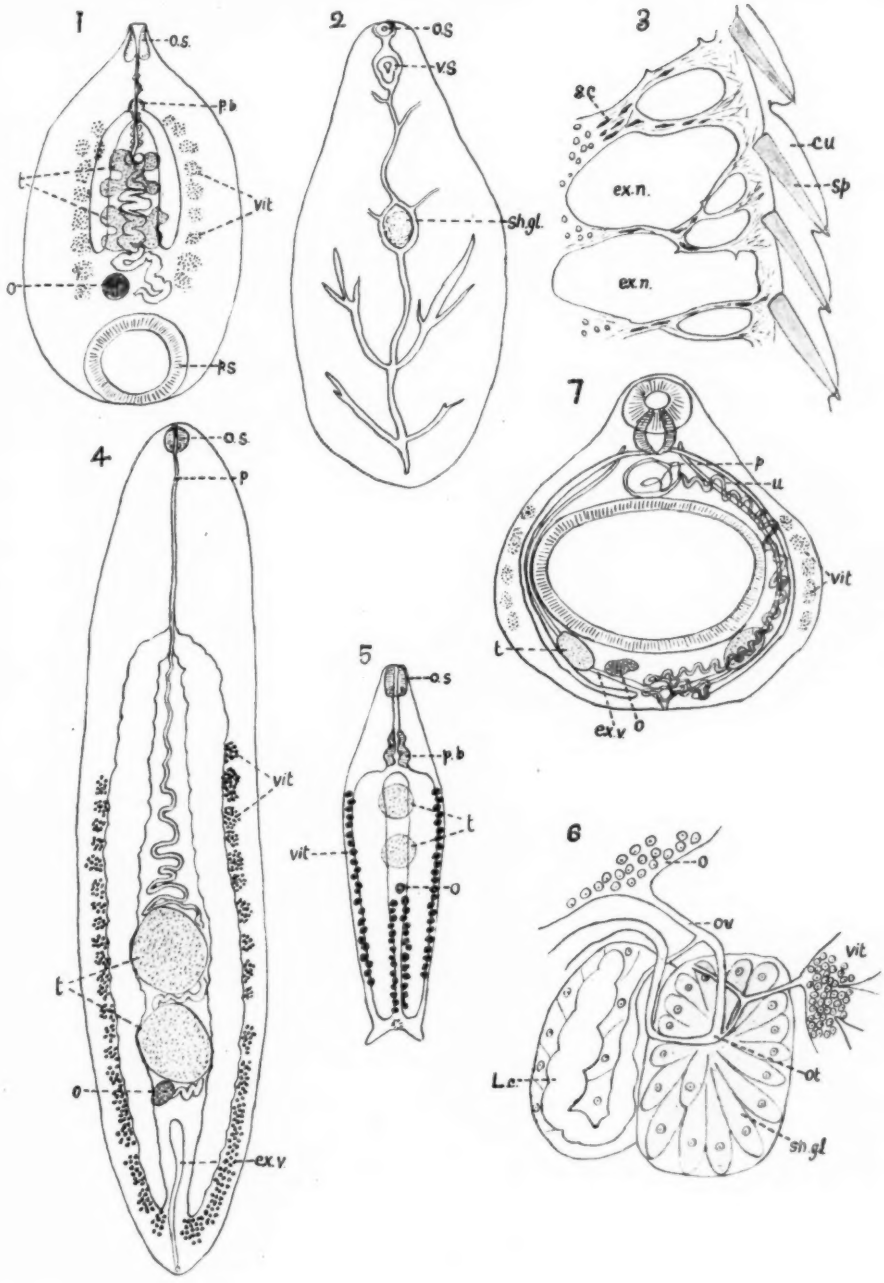
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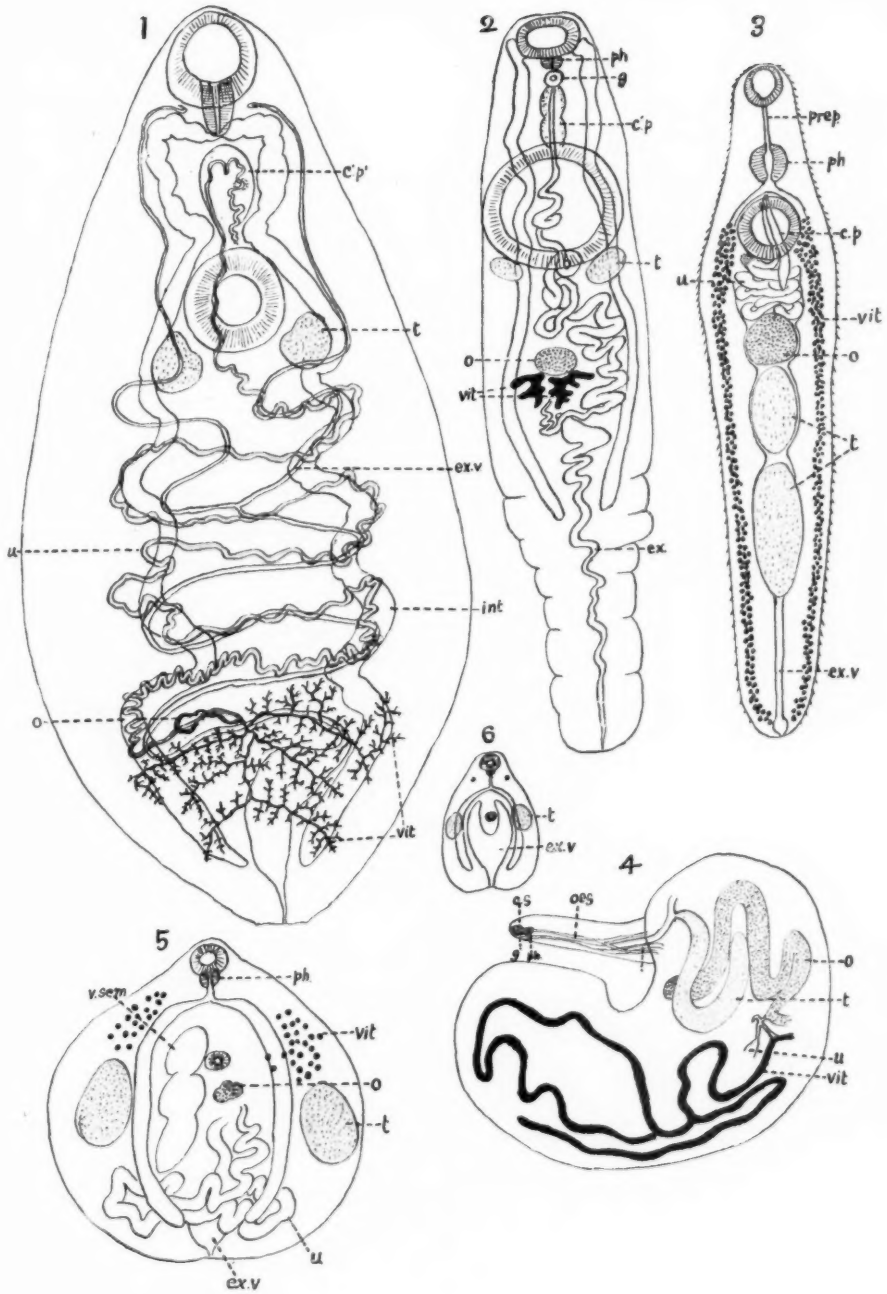
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ON THE CLASSIFICATION OF THE ASCARIDAE.

II. THE *POLYDELPHIS* GROUP; WITH SOME ACCOUNT OF OTHER ASCARIDS PARASITIC IN SNAKES.

By H. A. BAYLIS, M.A.

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(With 7 Text-figures.)

The Ascarids parasitic in snakes present considerable difficulties from the systematic point of view, owing to the incomplete character of many of the descriptions, and to the state of confusion which has long existed in the nomenclature. The writer has made an attempt, by examining such material as was available, and by comparing a number of the existing descriptions, to clear up some of this confusion. It was hoped also that such a comparative treatment would throw some light on the systematic value of the modifications of the female genital apparatus, which occur in some of these forms.

The typical female *Ascaris*, as is well known, possesses two uterine branches, commonly running backwards and parallel to each other from their point of origin in the common uterine tube. Retzius, in 1830, was the first to describe a multiplication of the uterine branches, occurring in an Ascarid from the python, subsequently redescribed and named *Ascaris anoura* by Dujardin (1845). In this form the uterus is divided into four, instead of two, branches, and Dujardin regarded it as representing a sub-genus, which he named *Polydelphis*, of the genus *Ascaris*. Since that time other forms having a four-branched uterus have been described from snakes. It was not, therefore, very surprising to the writer to discover, in 1916, an Ascarid from a snake showing a still further increase in the number of uterine branches. This was *A. boddaertii* Baird, in which the branches were found, on re-examination of the type, to be six in number. About the same time Gedoelst (1916) described a form, *Ascaris hexametra*, from a chamaeleon, in which a similar structure was seen; and more recently the writer has observed the same modification in two more Ascarids from snakes.

The Ascarids of snakes seem, as far as can be ascertained at present, all to belong to the sub-family Ascarinae. They show remarkably few characters which can be regarded as of more than specific value, in the present state of our knowledge of the Ascarid family as a whole. Most of them, however, appear to fall into two fairly well-marked groups, which may, provisionally at least, be treated as genera.

The first group consists of forms in which the uterus is of the primitive two-branched type, but with the vulva usually situated in the posterior region of the body, and rarely in front of the middle, its more usual position among the Ascarinae. These forms are further characterised by the presence of more or less well-developed interlabia between the main lips, and usually by having the cuticle at the bases of the main lips deeply grooved by an incision running in from the interlabium on either side.

The second group consists of species in which the uterus breaks up into more than two (four or six) branches, the vulva is situated, with few exceptions, in front of the middle of the body, and interlabia are absent. One species not from a snake, but from a chamaeleon, is included in this group.

Among the characters common to all, or most, of the species comprised in both groups are the general form of the spicules of the male, which have a tubular shaft and two broad membranous alae; the presence of simple dentigerous ridges on the inner surfaces of the lips; and the character of the ova, which are in all cases large, nearly spherical or of a much rounded oval shape, with a thick shell ornamented with granulations externally, and with the contents only segmenting at the time of laying. A further character which is not improbably universal is the presence of series of radiating digitiform processes on the anterior borders of the pulps of the lips. These processes are sometimes obvious in some specimens and hard to detect in others of the same species, and their visibility or otherwise evidently depends to a large extent on the state of preservation of the material and the technique employed in its examination. It is difficult, therefore, to make use of this character for systematic purposes.

The two main groups already referred to, and their component species, may now be diagnosed.

(1) **Ophidascaris,** n.g.

Generic Diagnosis. *Ascarinae*: Lips almost square, with more or less rounded angles, and generally as broad as long. Dorsal lip slightly smaller than ventro-lateral lips. Interlabia usually well-developed. From the interlabia deep transverse grooves in the cuticle run partially round the bases of the main lips towards their main axes. Oesophagus relatively short, without bulb or ventriculus. No oesophageal or intestinal caeca (the intestine, however, is frequently pushed up into an annular "caecum" round the posterior end of the oesophagus). Vagina and uterus run backwards from the vulva. Uterus with two parallel branches. Vulva usually behind middle of body, and genital organs (in both sexes) usually confined to the posterior region of the body, which often shows a fusiform thickening.

Genotype: *O. filaria* (Dujardin, 1845).

1. *Ophidascaris filaria* (Dujardin, 1845).

Ascaris filaria Dujardin (1845), pp. 177, 653.
" " Stossich (1896), p. 79.
" " Railliet and Henry (1910).
" *rubicunda*, ♂, Schneider (1866), p. 42; Text-fig.; Pl. I, fig. 8.
" " Stossich (1895), p. 35; Pl. VI, figs. 30, 31, 35.
" " " (1896), p. 35.
? " *infundibulicola* v. Linstow (1903), p. 108; Pl. V, figs. 1–2.

Length, male, up to 110 mm.; female up to 170 mm. Thickness, male, 1 mm.; female, 1·5 mm. Cuticular striations about 3 μ apart. Body cylindrical, greatly elongate, a little more tapering in front than behind. Lips (fig. 1) each bearing two papillae externally; the papillae of the dorsal lip with double terminations; ventro-lateral lips each with a large papilla towards the ventral and a smaller papilla towards the dorsal side. Dorsal lip with slightly emarginate

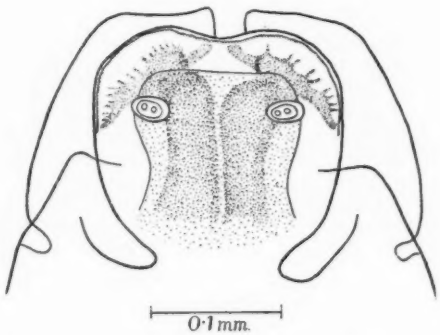


Fig. 1. *Ophidascaris filaria.* The head; dorsal view.

anterior border and rounded free angles. Free end of each lobe of pulp multiradiate, with an antler-like lobule directed laterally and posteriorly. Interlabia short, bluntly conical. Well-developed grooves at bases of lips. Marginal dentigerous ridges present. Oesophagus 5–7 mm. long, somewhat swollen posteriorly. Excretory pore at about 1·4 mm. from the anterior end. Tail of male bluntly conical. Spicules slightly unequal, rounded at the tip, measuring about 4–4·8 mm. in length. Width of spicules, including shaft and alae, 0·06–0·07 mm. Postanal caudal papillae six pairs, of which pairs 1–5 form an almost circular group on either side near the tip of the tail, the sixth pair being situated a little behind the cloaca, and having double terminations. Tail of female bluntly conical, about 0·3 mm. long. Vulva situated considerably behind middle of body, dividing the total length in the proportion of about

7 : 4[1]. Vagina simple, muscular, 0·15 mm. in diameter, sometimes running forward at first from the vulva, then doubling back with a sinuous course, and passing, a little behind the level of the vulva, into an oval swelling (measuring about 0·4 × 0·27 mm.). This is followed by a straight, unpaired portion of the uterus, about 4 mm. long, and with a maximum width of 0·4 mm. The uterus divides into two wide branches, which run posteriorly, parallel to each other, forming a few bends at first, and then becoming quite straight. Posteriorly these suddenly pass into two short, narrow, muscular ducts, which, after a course of about 0·7 mm., expand into fusiform swellings and then join the oviducts. The ovaries turn forward soon after their origin, the anterior limit of their coils being at about the level of the bifurcation of the uterus, from which point they return to the posterior end, and terminate at about 1·5 mm. in front of the anus. Ova nearly spherical, measuring 0·065–0·073 mm. in diameter.

Hosts: *Python molurus*, *P. reticulatus*, *P. sebae*, *P. spilotes*; also *Varanus* sp. (Zanzibar—in British Museum collection).

Geographical Range: Africa, India, Malay Peninsula and Archipelago, Australasia.

2. *Ophidascaris radiosa* (Schneider, 1866).

Ascaris radiosa Schneider (1866), p. 42; Text-fig.; Pl. I, fig. 9.
„ „ Stossich (1896), p. 36.

Length, male, 160 mm.; female, up to 270 mm. Maximum thickness 1·5 mm. The British Museum collection contains a single female specimen, 107 mm. long and 0·85 mm. thick, which is probably referable to this species. As Schneider did not describe the female beyond giving its size, this specimen will help to complete the description. It very closely resembles *O. filaria*. The lips, however (Fig. 2), show an important difference in outline. The dorsal lip is nearly square, with the anterior border more strongly emarginate than is the case in *O. filaria*, and the free angles much more acute. The ventral angles of the ventro-lateral lips project in a striking manner. The cephalic papillae are similar to those of *O. filaria*. The dentigerous ridges have not been seen (according to Schneider, they are present at some distance from the edge of the lip). The lobes of the pulp are multiradiate. Interlabia short. Well-marked grooves at bases of lips. Oesophagus about 4 mm. long. Tail 0·2 mm. long, ending in a short spike. Vulva situated within the posterior third of the body, dividing the total length in the proportion of 15 : 6·5. The vagina runs straight back, and the uterus has two wide branches. The whole of the female organs lie in the region between vulva and anus. Ova spherical, measuring 0·08 mm. in diameter.

The male, according to Schneider, has a slight caudal "bursa," two pairs of almost lateral postanal papillae near the tip of the tail, and a single row of

[1] Railliet and Henry (1910) give "$\frac{4}{7}$" from the posterior end, probably a *lapsus* for $\frac{4}{11}$. v. Linstow, for *A. infundibulicola*, states, probably by a clerical error, that the vulva is in front of the middle, dividing the body in the proportion of 4 : 7.

six preanal papillae on either side, passing anteriorly into a double row, "probably again becoming single."

HOST: *Bitis gabonica.*

DISTRIBUTION: Africa.

3. *Ophidascaris obconica* (Baird, 1860).

Ascaris obconica Baird (1860), p. 447.
" " Baird (1861), p. 229.
" " Örley (1882), p. 310.
" " Stossich (1896), p. 80.
" " Baylis (1916), p. 413; Text-figs. 1–3.

This species has already been fairly fully re-described by the writer (1916). The following is a short summary of its chief characters.

Length up to 52 mm. (female); males slightly smaller. Maximum thickness 2 mm. A fusiform thickening of the posterior region of the body. Cuticular

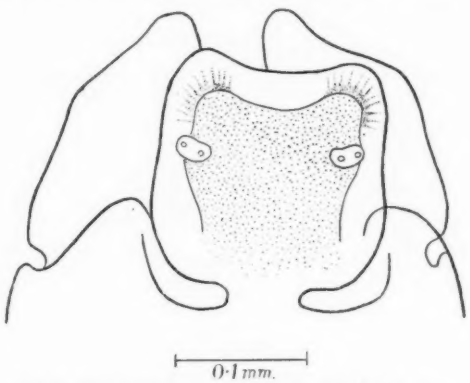


Fig. 2. *Ophidascaris radiosa.* **The head; dorsal view.**

striations 4·2 μ apart. Lips almost square, with an indentation in the anterior margin, and with the free angles rounded. Dentigerous ridges marginal. Interlabia small, conical. Well-marked grooves at bases of lips, almost meeting in middle line of lip. Oesophagus 2–3 mm. long. Tail in both sexes very blunt, without terminal spike. Spicules of male 2·48 mm. long and 0·08 mm. wide. Four pairs of postanal papillae, near tip of tail, and about 40 pairs of preanal papillae. Vulva in posterior third of body, dividing the total length in about the proportion of 12 : 5. Vagina runs forward, passing into the uterus a little in front of the vulva; uterus then bends back and gives off two branches which run, with many convolutions, parallel to each other towards the posterior end. Ova spherical, 0·1 mm. in diameter, with coarsely granulated shell.

HOST: *Helicops angulatus.*

LOCALITY: Brazil.

4. *Ophidascaris mombasica*, sp.n.

Length, female, up to 100 mm. (The only male available is not quite complete.) Maximum thickness, male, 1·7 mm.; female, about 2 mm. Cuticular striations exceedingly fine. Body thickest in the posterior quarter or third of its length; also slightly thickened near the anterior end; the middle region slender. Head up to about 0·45 mm. in diameter. Lips (Fig. 3) large, rather broader than long. Dorsal lip with two simple papillae, placed near the anterior margin. Ventro-lateral lips each with a lozenge-shaped papilla, with double termination, towards the ventral side, and a small, simple papilla towards the dorsal side, the latter nearly marginal. Interlabia massive, about half the length of the lips. Grooves at bases of lips well-developed. Dentigerous ridges marginal, with large teeth, and with an indentation in the middle anteriorly. Oesophagus about 3 mm. long. Tail of male 0·25 mm. long, with a very short terminal spike. Spicules blunt at the tip, measuring 3·7 mm. in length, and, with the alae, 0·09 mm. in width. Postanal caudal papillae five

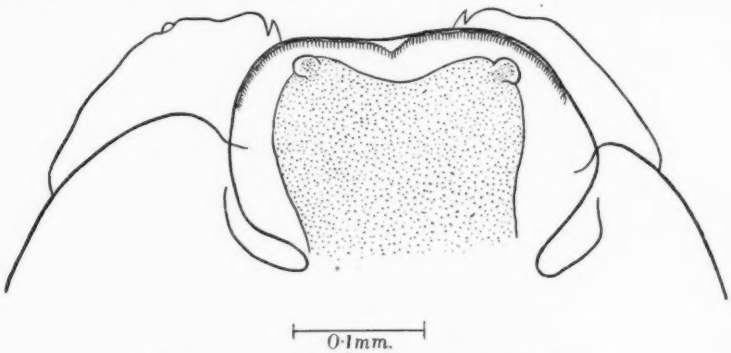


Fig. 3. *Ophidascaris mombasica.* The head; dorsal view.

pairs, of which four pairs form a group near the tip of the tail, two being large and lateral, two smaller and ventral. The papillae of the posterior ventral pair have double terminations. The fifth pair, which are widely separated from the rest, and lie near the corners of the cloacal aperture, are also double papillae, but not of very large size. Preanal papillae about 35 pairs, the intervals between them gradually becoming longer anteriorly. Tail of female very short (0·2 mm.) and blunt. Vulva situated at about the beginning of the posterior quarter of the body. Vagina runs forward a little, then doubles back, widening gradually into the uterus, which has two very wide branches running back almost to the tail. The coils of the ovarian tubes extend forward to a point a short distance in front of the vulva, and backward nearly to the posterior end of the body. Ova roundish-oval, measuring 0·0875–0·1 mm. × 0·07–0·0825 mm.

Host: *Psammophis subtaeniatus.*

Locality: Mombasa.

The following forms probably also belong to this genus:

5. *Ophidascaris gestri* (Parona, 1890).

Ascaris gestri Parona (1890), p. 768; Pl. III, figs. 7–8.
" " Stossich (1896), p. 53.

Length, male, 55–67 mm.; female, 55–78 mm. Maximum thickness, 1 mm. Cuticular striations fine. Body elongate and slender, not tapering much at the extremities. Dorsal lip with two small rounded marginal papillae. Ventrolateral lips with two [? two each] small papillae on their margins anteriorly, and with an indentation on each side at their bases. Tail of female very short, with a terminal spike. Vulva in anterior third of body. [Uterus?] Tail of male with a terminal spike. Papillae small and sessile, two pairs postanal and ten pairs preanal. Spicules long, equal, with rounded tips.

Host: *Tropidonotus piscator* [*T. quincunciatus*].
Locality: Northern Tenasserim.

6. *Ophidascaris papillifera* (v. Linst., 1897).

Ascaris papillifera v. Linstow (1897), p. 281; Pl. XXI, figs. 1–3.

Length, male, 38·2 mm.; female, 55 mm. Thickness, male, 0·75 mm.; female, 1·1 mm. Cuticular striations 0·039 [? 0·0039] mm. apart. Body much attenuated in front. Tail in both sexes very short and rounded. Lips almost circular, with marginal dentigerous ridges. Dorsal lip somewhat longer than broad, with narrow base, and bearing in front two large, oval, diagonally-placed papillae. Pulp with two lobules projecting forwards and inwards. Interlabia short, conical and rounded. Tail of male $\frac{1}{323}$ of total length. Six pairs of postanal papillae, of which one small pair is close to the cloaca and near the mid-ventral line, and one very large pair just behind these and more laterally placed. Preanal papillae 32–34 pairs, extending forward to 1·78 mm. from the caudal end, larger and closer together behind, becoming smaller and more widely spaced in front. Spicules 4·74 mm. long and 0·062 mm. wide. Tail of female $\frac{1}{324}$ of total length. [Vulva? Uterus?] Ova with very thick, closely stippled shell, measuring 0·073 × 0·065 mm.

Hosts: Snakes (*indet.*).
Locality: Bismarck Archipelago.

7. *Ophidascaris solitaria* (v. Linst., 1903).

Ascaris solitaria v. Linstow (1903), p. 109; Pl. V, fig. 3.

Length (immature female), 44 mm. Thickness, 0·81 mm. Dorsal lip oval, broader than long (0·14 mm. × 0·078 mm.). Dentigerous ridges nearly marginal. Interlabia low, pyramid-shaped. Oesophagus $\frac{1}{15}$ and conical tail $\frac{1}{259}$ of total length. [Vulva? Uterus?] Male unknown.

Host: *Dipsadomorphus dendrophilus*.
Locality: Siam.

8. *Ophidascaris naiae* (Gedoelst, 1916).

Ascaris naiae Gedoelst (1916), p. 3.

Length, male, 62 mm.; female, 56·7 mm. Thickness, 1–1·07 mm. Cuticular striations 2·5–3 μ apart. Body cylindrical, more tapering in front than behind. Lips square, with rounded angles. Dorsal lip with two papillae, ventro-lateral lips each with one papilla. Dentigerous ridges present. Interlabia small, narrow, with rounded extremity, $\frac{1}{3}$ of length of lips. Oesophagus 2·9 mm. long, surrounded by the nerve-ring within its anterior fifth. Tail of male 0·2 mm. long, with six postanal papillae (four ventral, two sub-dorsal); preanal papillae 35 pairs. Spicules slightly unequal in length (5·04 mm. and 4·64 mm.), with rounded tips. Tail of female 0·24 mm. long, with conical tip. Vulva in middle of body. [Uterus?] Ova elliptical, with thick, finely punctate shell, measuring 0·08 × 0·072 mm.

Host: *Naja nigricollis*.

Locality: Belgian Congo.

9. *Ophidascaris intorta* (Gedoelst, 1916).

Ascaris intorta Gedoelst (1916), p. 4.

Length (immature females) 100–110 mm. Thickness 0·736 mm. Cuticular striation not visible. Lips large, quadrangular, with minute, marginal dentigerous ridges. Interlabia small, triangular, with rounded extremity. Dorsal lip with two papillae, ventro-lateral lips with one each. Pulp with a flabelliform lobule directed forwards and outwards on either side, the free edge of which bears very numerous sharp digitations. Of these the outermost is the largest, and is directed backwards. Oesophagus $\frac{1}{27{\cdot}6}$ of total length, surrounded by the nerve-ring within its anterior fifth. Excretory pore at 1·07 mm. from anterior end. Tail blunt, rounded, 0·18 mm. long, with terminal spike 0·03 mm. long. Vulva in anterior third of body. [Uterus?] Genital organs lie behind the level of the vulva.

Host: *Bitis* sp.

Locality: Belgian Congo.

(2) **Polydelphis** Duj., 1845.

Polydelphis Dujardin (1845), p. 221 (as sub-genus of *Ascaris*).

Generic Diagnosis. *Ascarinae*: Lips oblong, or more or less hexagonal, in outline, frequently longer than broad; usually broader at the base than at the free edge. Dorsal lip usually smaller than ventro-lateral lips. Two simple or double papillae on dorsal lip; one large simple or double papilla towards ventral side of each ventro-lateral lip. Pulp of ventro-lateral lips sometimes asymmetrical. Interlabia absent. No grooves at bases of lips. Oesophagus usually short, without bulb or ventriculus. A rudimentary intestinal caecum

sometimes present. Vulva usually in anterior region of body, rarely behind the middle. Vagina and uterus run posteriorly, the latter giving off either four or six parallel branches.

Genotype: *P. anoura* Dujardin, 1845. (= *Ascaris* (*P.*) *pythonis* Retzius, of authors.)

The species may be divided into two sections, according to the number of uterine branches, as follows:

Section I. Forms with four-branched uterus.

1. *Polydelphis anoura* Duj., 1845.

Ascaris (*Polydelphis*) *anoura* Dujardin (1845), p. 221.
Ascaris attenuata Molin, 1858, of Stossich (1896), p. 77 (in part).
„ „ „ (♂) of v. Linstow (1899), p. 6.
„ *pythonis* Retzius, 1830, of Railliet and Henry (1910) (in part).
„ (*Polydelphis*) *pythonis* (Retzius, 1830) of Gedoelst (1916), p 7.

Length, male, up to 116 mm.; female, up to 144 mm. Thickness, male, 2·15 mm.; female, 2·5–2·8 mm. Cuticular striations distinct, 4–10 μ apart. Body tapering in front, thick posteriorly. Lateral fields wide, more transparent than the rest of the cuticle. Lips small, rather longer than broad, nearly straight in front. Dorsal lip with two large double papillae; ventro-lateral lips each with one large double papilla towards the ventral side. Dentigerous ridges marginal, with minute teeth. Oesophagus[1] relatively long (10 mm.). No intestinal caecum. Tail of male blunt, 0·35 mm. long, with a small terminal spike. Spicules equal, conically pointed, 10·5 mm. long. Caudal papillae, two pairs postanal and 25 pairs preanal. Vulva at a little less than one-third of the total length from anterior end. Vagina passes gradually into the undivided portion of the uterus, which has a fusiform swelling about its middle. Length of this part of the uterus, together with vagina, about 30 mm. The four uterine branches relatively very short, originating in an oval swelling or reservoir, and running straight backward to end suddenly in narrow muscular canals about 3 mm. long. The latter are separated from the oviducts by globular or fusiform swellings. The four genital tubes bend forward soon after the termination of the uterine branches, the ovaries forming many coils about the intestine, and occupying the greater part of the posterior region of the body between vulva and anus. Ova nearly spherical, measuring 0·066–0·072 mm. in diameter.

Hosts: *Python molurus*, *P. sebae*. Said also to occur in *Bitis arietans*, *Drymobius bifossatus*, [= *Coluber lichtensteinii*], *Coluber corais*, *Zamenis constrictor*, ? *Coronella* [*Ophibolus*] sp.

Geographical Range: India, Africa, ? America.

[1] Dujardin's statement that the oesophagus is followed by a narrow ventriculus seems to be erroneous.

Note on the name P. anoura.

This form does not appear to have been named by Retzius (1830), although it was first described by him. He refers to it merely as an Ascaris from *Python bivittatus*. Dujardin (1845), who re-described it, makes no reference to Retzius' description, but names it, as a new species, *anoura*. Railliet and Henry (1910) make the somewhat misleading statement that Retzius, in 1848, recognised his species as identical with *A. anoura* Dujardin, 1845. The fact appears to be that Creplin (1848) published a German translation of Retzius' description, adding footnotes of his own (signed "Cr."), in one of which he observes that Retzius' and Dujardin's species are identical, and appears to accept Dujardin's name. It seems, therefore, that the correct specific name of the form just described is *anoura*, and that the name *pythonis* has no claim to priority. How the apparently fictitious name *A. pythonis* has crept into the literature the writer is unable to discover. Possibly there has been some confusion between the *Ascaris* from a python and the *Bothriocephalus pythonis* described by Retzius in the same year and journal.

2. *Polydelphis attenuata* (Molin, 1858).

Ascaris attenuata Molin (1858), p. 147.
„ *rubicunda* (♀) Schneider (1866), p. 42.
„ *attenuata* Molin, 1858, of Stossich (1896), p. 77 (in part).
„ „ (♀) Molin, of v. Linstow (1899), p. 6.
„ *pythonis* Retzius, 1830, of Railliet and Henry (1910) (in part).
„ (*Polydelphis*) *attenuata* (Molin, 1858) of Gedoelst (1916), p. 5.

Length, male, up to 190 mm.; female, up to 258 mm. Thickness, male, up to 2 mm.; female, up to 3 mm. Cuticular striations 3 μ apart. Body tapering anteriorly, stout posteriorly. Lips (Fig. 4) small, oblong, somewhat longer than broad, with anterior border slightly emarginate and free angles rounded. Dorsal lip bears two papillae with double terminations, ventro-lateral lips each one double papilla towards the ventral side. Dentigerous ridges marginal, with very minute teeth. Oesophagus relatively long, $\frac{1}{11}$ to $\frac{1}{9}$ of total body-length. No intestinal caecum. Nerve-ring at about the anterior $\frac{1}{11}$ of oesophagus. Excretory pore a little behind nerve-ring. Tail of male conical, $\frac{1}{229}$ of total length. Spicules equal, 7–9 mm. long, with blunt tips. Caudal papillae, five pairs postanal (two sub-ventral, three lateral), 36 pairs preanal. Genital tube of male extends as far forward as the anterior third of the body. Posterior end of female very blunt, with small terminal spike. A pair of caudal papillae at 0·175 mm. from the extremity. Vulva at about the anterior fifth of the body. Vagina sinuous, widening gradually into the undivided portion of the uterus, from which it is not clearly marked off externally. The anterior part of the uterus runs straight back to a point about 35 mm. from the anterior end of the body, where it gives off four wide branches. These run straight back and

narrow suddenly behind into short muscular canals, about 1·5 mm. long, followed by small swellings of fusiform, oval or globular shape, apparently functioning as receptacula seminis. These mark off the uterine tubes from the oviducts, which, with the ovaries, are thrown into many convolutions, extending backwards nearly to the posterior end of the body, and then running forwards to a point about 5 mm. behind the vulva. Ova of roundish-oval shape, measuring 0·075–0·090 mm. × 0·065–0·070 mm.

HOSTS: *Python molurus, P. sebae, ? P. reticulatus, Bitis arietans.*

GEOGRAPHICAL RANGE: India, Africa, ? Malay region.

3. *Polydelphis oculata* (v. Linst., 1899).

Ascaris oculata v. Linstow (1899), p. 6; Pl. I, fig. 7.
„ (*Polydelphis*) *oculata* Linst., 1907, of Railliet and Henry (1910).

Length, male, 58 mm.; female, 63 mm. Thickness, male, 2 mm.; female, 2·41 mm. Body tapering in anterior third, stout and cylindrical posteriorly.

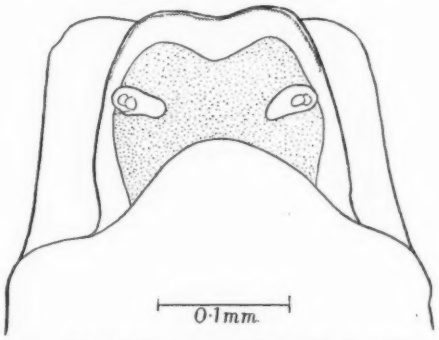


Fig. 4. *Polydelphis attenuata.* The head; dorsal view.

Lips nearly square, with free angles rounded. Dorsal lip slightly broader than long (0·18 × 0·14 mm.). Cephalic papillae very large; two on dorsal lip. Each lobe of pulp of lip gives off an inward projection. Dentigerous ridges present. Oesophagus 6 mm. long. No intestinal caecum. Tail of male $\frac{1}{265}$ of total length, with a small terminal digitiform appendage. Spicules 5·4 mm. long. Caudal papillae, two pairs postanal, six pairs preanal. Tail of female $\frac{1}{53}$ of total length, blunt. Vulva at anterior third of body. Vagina at first simple, slender, sinuous, with a fusiform swelling near its origin, giving off, at 11 mm. from its origin, four wide branches 2 cm. long, lying in the same plane, and running backwards. Ova 0·060–0·067 mm. × 0·055–0·060 mm. Embryos on hatching measure, according to Railliet and Henry, 0·4–0·425 mm. long.

HOSTS: *Python reticulatus, P. sebae.*

GEOGRAPHICAL RANGE: Malay region, Africa.

SECTION II. Forms with six-branched uterus.

1. *Polydelphis quadricornis* (Wedl, 1862).

Ascaris quadricornis Wedl (1862), p. 469; Pl. II, figs. 17–19.
„ „ Stossich (1896), p. 50.
? *Ascaris quadrangularis* Schneider (1866), p. 43; Text-fig.; Pl. I, fig. 10.
? „ „ Stossich (1896), p. 25.
? „ *quadrilobata* v. Linstow (1908), p. 21; Pl. IV, fig. 1.

Although Wedl expressly states that this species has a four-branched uterus, the writer, after examining several sets of specimens which in other respects agree very closely with the characters given for *A. quadricornis*, is inclined to believe that the original description, in this particular, is faulty. Should this supposition ultimately prove to be incorrect, the form here described, which seems to be fairly common in the puff-adder, is probably a new species.

Size of mature specimens very variable. Length, male, up to 130 mm.; female, 70–155 mm. Thickness 2–3 mm. Body tapering in front, less so behind.

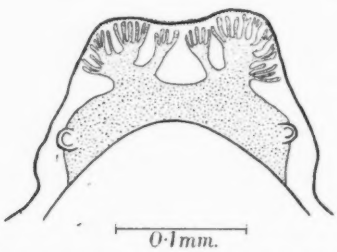


Fig 5. *Polydelphis quadricornis*. Dorsal lip, viewed from the exterior.

Lips somewhat hexagonal[1] in outline, broader at the base than at the free end. Dorsal lip (Fig. 5) with two small simple papillae near the lateral borders; ventro-lateral lips each with one large, lozenge-shaped papilla towards the ventral side. Pulp of dorsal lip sends out four lobes anteriorly, the outer lobe on each side large, the inner smaller. The outer lobes give off a large number of radiating processes, the inner lobes only four or five. Pulp of ventro-lateral lips asymmetrical, extending further forward laterally than ventrally; radiating processes present as in the dorsal lip. Dentigerous ridges marginal, with very small teeth. Oesophagus relatively short, about $\frac{1}{26}$ to $\frac{1}{18}$ of the total body-length. Many specimens have a short intestinal caecum running forward beside the base of the oesophagus, but this feature is apparently not constant. Tail in both sexes very short and rounded, with a small terminal spike. Tail of male with slight cuticular alae. Spicules short (about 1·75 mm.). Postanal papillae, five pairs—a group of four pairs near the tip of the tail (two lateral

[1] Wedl's figure of the lip (1862, Pl. II, fig. 19) indicates rather a square shape, but this may perhaps be due to the fact that, as stated, the lip is drawn from the inner aspect.

and two ventral) and a pair of large papillae, probably double, close behind and at the sides of the cloacal aperture. Preanal papillae at least 50 pairs. Vulva slightly in front of the middle of the body, in an annular constriction which is usually plainly visible to the naked eye. Vagina simple, muscular, about 4 mm. long in a 150 mm. specimen, passing into an undivided portion of the uterus, which gradually widens and, after a course of about another 4 mm., gives off six wide, thin-walled branches running back nearly straight for a distance of about 24 mm. These are followed by short, narrow, muscular canals, describing an **S**-shaped curve, and separated from the ovarian tubes by small oval swellings. The coils of the ovarian tubes extend backward nearly as far as the anus, and forward to the level of the vulva. In smaller specimens the measurements given for the female organs are, of course, proportionately smaller. Ova roundish-oval in shape, measuring 0·095–0·1 mm. × 0·0875–0·0925 mm.[1], with shell 0·0075 mm. thick.

Hosts: *Naja haje*, *N. nigricollis*, *Bitis arietans*, *Pseudaspis cana*, *Crotalus* sp.?

Geographical Range: Africa, ? Brazil.

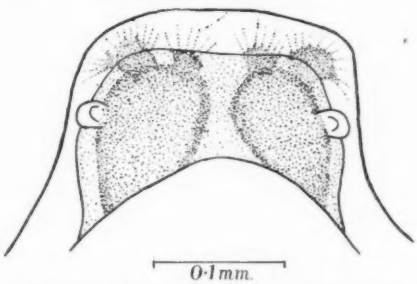


Fig. 6. *Polydelphis boddaertii*. Dorsal lip, viewed from the exterior.

2. *Polydelphis boddaertii* (Baird, 1860).

Ascaris boddaertii Baird (1860), p. 447.
„ „ Baird (1861), p. 229.
„ „ Örley (1882), p. 310.
„ „ Stossich (1896), p. 78.
Polydelphis boddaertii Baylis (1916), p. 416.

Length (female) 90 mm. Lips quadrangular, broader than long. Dorsal lip (Fig. 6) with two simple papillae; ventro-lateral lips each with one papilla. Pulp sends out radiating processes in front (vaguely-defined in the type-specimen), apparently from two main lobes on each side of the lip, the outer lobes being larger than the inner, as in *P. quadricornis*. Dentigerous ridges not seen. Oesophagus 4 mm. long. No intestinal caecum. Tail 0·4 mm. long, blunt, apparently without terminal spike. Vulva behind middle of body, dividing it in the proportion of 5 : 4. Vagina, together with undivided portion of uterus,

[1] 0·064 mm. × 0·054 mm., according to Wedl.

about 7 mm. long, running posteriorly, and ending in an oval uterine chamber which gives off, laterally and posteriorly, six wide branches. These follow a sinuous course, parallel to each other, to within about 13 mm. from the posterior end of the body. The ovarian tubes turn forward shortly after their origin, and their coils extend anteriorly for a short distance in front of the vulva. Male unknown. Ova roundish-oval, measuring 0·088–0·095 mm. × 0·075–0·080 mm., with shell about 0·007 mm. thick.

HOST: *Drymobius boddaertii*.
LOCALITY: West Indies.

3. *Polydelphis hexametra* (Gedoelst, 1916).

Ascaris hexametra Gedoelst (1916), p. 9.

Length, male, 51–55 mm.; female, 68·5–85 mm. Thickness, male, 1·3 mm.; female, 1·45 mm. Cuticular striation very fine, visible only under high powers. Body cylindrical, equally tapering at both ends. Lips sub-equal. Dorsal lip trapezoidal, with anterior angles rounded, bearing two lateral papillae. Ventro-lateral lips each with one papilla. Pulp undivided. Dentigerous ridges minute. Oesophagus $\frac{1}{22}$ to $\frac{1}{20}$ of total body-length. No intestinal caecum. Nerve-ring in anterior fifth or quarter of oesophagus. Excretory pore at 0·83–0·85 mm., and a pair of sessile cervical papillae at 0·975 mm., from the anterior extremity. Tail short, blunt, with small terminal spike. Tail of male with six pairs of postanal papillae—two pairs at about the anterior third of the tail (the more anterior of these sub-median); two large pairs at about $\frac{2}{3}$ of the length of the tail; one very small, sub-median pair and one larger, lateral pair a little in front of the tail-spike. Spicules unequal, measuring 0·91 mm. and 0·83 mm. in length, with rounded tips. Coils of male genital tube do not extend anteriorly beyond the middle of the body. Tail of female straight, $\frac{1}{152}$ of the body-length. Vulva in front of the middle of the body. Vagina, together with undivided portion of uterus, about 2·76 mm. long. Uterus gives off six branches 14 mm. long, continued as narrow muscular tubes, 0·4–0·45 mm. long, which pass into the oviducts. Most of the coils of the ovarian tubes extend backward to the posterior $\frac{1}{10}$ of the body, a few folds reaching forward to 1·9 mm. in front of the vulva. Ova nearly spherical, mean measurements 0·08 mm. × 0·072 mm. Shell thick, with smooth [?] surface.

HOST: *Chamaeleon dilepis*.
LOCALITY: Belgian Congo.

4. *Polydelphis waterstoni*, sp.n.

Length (female) up to 110 mm. Thickness, 1·45 mm. Cuticular striation not apparent. Body slender, more tapering in front than behind. Diameter of head 0·3 mm. Lips rather broader than long, widest at the base, somewhat

hexagonal in outline, with straight anterior border. Dorsal lip with two simple lateral papillae; ventro-lateral lips each with one papilla towards the ventral side. Dentigerous ridges nearly marginal, with very small teeth. Oesophagus about $\frac{1}{27}$ of total length, with a maximum thickness (near the posterior end) of 0·35 mm. A short intestinal caecum present, about 0·4 mm. long, springing forward from a little behind the junction of oesophagus and intestine. Excretory pore at 0·95 mm., and nerve-ring at 0·8 mm., from the anterior end. A pair of cervical papillae, not prominent, just behind the nerve-ring. Vulva behind the middle of the body, dividing the total length in the proportion of 6 : 5. Vagina runs back as a muscular tube for about 3 mm. before widening into the uterus, which gives off six branches, running parallel to each other posteriorly. The coils of the ovarian tubes extend back to 13 mm. from the caudal end, and forward to a little behind the vulva. Ova nearly spherical, measuring 0·08–0·1 mm. in diameter. Male unknown.

Host: *Zamenis gemonensis*, var. *caspius*.

Locality: Macedonia.

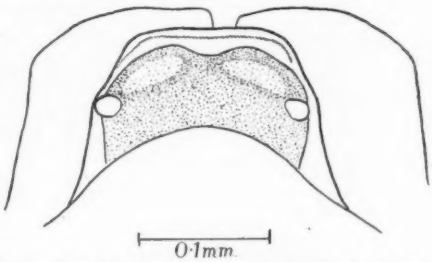


Fig. 7. *Polydelphis waterstoni*. The head; dorsal view.

Note. In the foregoing account no attempt has been made to give a complete list of the Ascarids of snakes. Some forms recorded under the name of *Ascaris* have been intentionally omitted, which cannot be assigned to either of the genera *Ophidascaris* and *Polydelphis*, or of which no description exists which is adequate for the determination of their position.

The nomenclature of the hosts has been, as far as possible, revised and corrected with the aid of Boulenger's Catalogue of the Snakes in the British Museum (1893–1896), and the writer's best thanks are also due to Dr Malcolm Smith for his help in this direction. Synonyms have been eliminated from among the names of hosts, except where it seemed an advantage to retain them, in which cases they have been enclosed in square brackets.

REFERENCES.

Baird, W. (1860). Description of some New Species of Intestinal Worms in the Collection of the British Museum. *Proc. Zool. Soc.*, London, p. 446.

—— (1861). Description of some New Species of Intestinal Worms in the Collection of the British Museum. *Ann. and Mag. Nat. Hist.* (3) vii. p. 228.

Baylis, H. A. (1916). The Types of the Species of *Ascaris* described by Baird. *Parasitology*, viii. 4, p. 411.

Creplin, F. C. H. (1848). See Retzius, A. (1848).

Dujardin, F. (1845). *Histoire naturelle des Helminthes.* Paris.

Gedoelst, L. (1916). Notes sur la Faune parasitaire du Congo belge. *Rev. Zool. Afric.* v. 1.

Linstow, O. von (1897). Nemathelminthen gesammelt von Herrn Prof. Dr F. Dahl im Bismarck-Archipel. *Arch. f. Naturg.*, Berlin, lxiii. Bd. I. p. 281. Plates XXI–XXII.

—— (1899). Nematoden aus der Berliner Zoologischen Sammlung. *Mt. Zool. Mus.*, Berlin, i. 2, p. 6. Plates I–VI.

—— (1903). Parasiten, meistens Helminthen, aus Siam. *Arch. f. mikr. Anat.*, Bonn, lxii. p. 108. Plate V.

—— (1908). Schultze's Reise in Westl. u. Zentr. Sudafrika, ii. Helminthes. Nematoden u. Acanthocephalen. *Denk. Med. Ges.*, Jena, xiii. p. 19. Plate IV.

Molin, R. (1858). Prospectus Helminthum quae in prodr. faunae helminthol. Venet. continentur. *Sitz. k. Akad. Wiss.*, Wien, xxx. p. 127.

Örley, L. (1882). Report on the Nematodes in the Possession of the British Museum. *Ann. and Mag. Nat. Hist.* (5) ix. p. 301.

Parona, C. (1890). Sopra alcuni Elminti di Vertebrati Birmani, etc. *Ann. Mus. Civ. Stor. Nat. Genova*, (2) vii. (xxvii.) p. 765. Plate III.

Railliet, A. and Henry, A. (1910). Sur quelques Helminthes du "Python sebae" (Gmelin). *Bull. Soc. Path. exot.*, Paris, iii. 2. p. 94.

Retzius, A. (1830). Beskrifning öfver en ny art Spolmask, funnen hos Python *bivittatus*, etc. *K. Vetensk.-Acad. Handl.*, Stockholm, 1829 (pub. 1830) p. 103. Plate V.

—— (1848). Beschreibung einer neuen Spulwurm-Art, gefunden im *Python bivittatus*, etc. [Translated, with notes, by Creplin.] *Arch. f. Naturg.*, Berlin, xiv. Bd. I, p. 166. Plate VI.

Schneider, A. (1866). Monographie der Nematoden. Berlin.

Stossich, M. (1895). Notizie elmintologiche. *Boll. Soc. Adriat. Sci. Nat.*, Trieste, xvi. p. 33. Plates IV–VI.

—— (1896). Il Genere *Ascaris* Linné. *Ib.* xvii. p. 9.

Wedl, K. (1862). Zur Helminthenfauna Ägyptens. *Sitz. k. Akad. Wiss.*, Wien, xliv. Abth. I. p. 463. Plates I–III.

ON THE CYSTS OF A HITHERTO UNDESCRIBED SPECIES OF *EIMERIA* IN HUMAN STOOLS.

By E. P. SNIJDERS.

(*From the Pathological Laboratory, Medan, East Coast of Sumatra.*)

(With 1 Text-figure.)

I.

Mr C., who has lived for ten years in the tropics, was under the care of Dr de Jong and myself for chronic amoebic dysentery. Some five years ago he suffered from an abscess of the liver, readily cured after operation. Before this abscess he had never noticed any dysenteric symptoms, but afterwards he often had mild, or sometimes more severe, attacks of recurring dysentery, most times without fever. During the attacks, the bloody slime contained a great number of amoebae, principally of *Entamoeba histolytica* and containing erythrocytes. With an "emetine cure" the attacks regularly subsided; but between two attacks the patient produced constantly a great quantity of so-called "*minuta* forms" and 4-nucleate ("*tetragena*") cysts of this species in the faeculent parts of the stools. After an emetine treatment I often found a rather sudden increase in the number of cysts (always measuring from 11·5–13·5 μ), sometimes to a tremendous amount[1]. Several times we tried to get rid of the amoebae by a cure with emetine bismuthous iodide, but without success, so the patient stayed under observation and regularly sent us his stools for control.

Now in the beginning of April, 1920, I received his stool once more, half-an-hour after he had passed it into a clean Petri dish. (This is our routine method to prevent contamination.) The stool was faeculent and pultaceous and contained no mucus or blood.

I found a few "*minuta* forms," but to my astonishment many round cysts, occurring uniformly in all parts of the faeces, but of larger dimensions than any that I had ever seen in human stools.

Without difficulty one could see that most of these cysts contained four whetstone-shaped structures strongly suggesting spores. These spores, in their turn, each enclosed two bodies—probably sporozoites; so on the whole I felt sure that I had the oöcysts of some species of *Eimeria* before me. Some of the oöcysts, however, were not completely differentiated; very few of them contained only one undifferentiated sphere showing a honeycomb structure, some others only two spores.

[1] Indeed for some time he formed my regular supply of cysts.

The oöcysts were colourless, transparent, and spherical. Their diameter was between 40 μ and 48 μ, most of them measuring 45 μ. The capsule was, as far as could be seen, composed of two layers, quite transparent; the inner one being the proper, homogeneous, well-defined wall, the outer appearing as an ill-defined mucous layer which was often difficult to detect.

The whetstone-shaped spores had a length varying from 17–20 μ and a breadth of 7–8 μ. The wall of the spore (sporocyst) was sharply defined except at certain spots on its outer surface where it appeared rough, possibly indicating the remains of a mucous outer layer. In general the ends of the spores were acute, but sometimes they were rather blunt.

It was rather difficult to see the form of the sporozoites, as will be clear from the drawings. They seemed to be rather slender, one end being more obtuse than the other, and to have these obtuse ends directed towards opposite poles. I could not detect nuclei with certainty and the "crystalline" small bodies observed by Dobell (1919) in *E. oxyspora* seemed to be absent.

Of an oöcystic residual body only vestiges seemed sometimes present. On the contrary, however, sporocystic residua were quite evident in the form of one or two highly refractile spherical bodies of varying, but rather small, size.

Sometimes the sporozoites had a "fixed," coagulated appearance, with some bursts, suggesting necrosis and disintegration of their coagulated bodies. Suspended in a 2 per cent. eosin solution (with 0·9 per cent. NaCl) these forms readily stained red, confirming the fact that these sporozoites were dead already.

Trying to obtain stained and durable preparations I met with difficulties. With the Heidenhain stain (after moist fixation in the warm fluid of Schaudinn) I did not succeed in obtaining any differentiation. One saw only the outline of the oöcysts and sometimes of the sporocysts, but nothing of their contents. In counterstaining with eosin, however, the bodies of the sporozoites stained red. But further details were not obtainable, at least not distinctly and constantly enough to rely on.

As of course these oöcysts puzzled me greatly, I afterwards made repeated examinations of this patient's stools, but I never succeeded in finding them again.

I may stipulate here that I have never found oöcysts of this kind in other patients, although I have made between 1000 and 2000 stool examinations a year for a period of six years, nor have I ever seen them in animal faeces. The only coccidial parasites I frequently meet with are those of *Eimeria stiedae* in rabbits and *Isospora bigemina* in cats[1].

Trying to identify this *Eimeria* I consulted Dobell's critical and exhaustive epitome on coccidia parasitic in man (1919), and for a moment thought it

[1] They are quite like the European forms; only it struck me that the dimensions of the cysts of *Eimeria stiedae* are smaller (length 30 μ (25–35 μ), breadth 12–20 μ) than is generally admitted in Europe.

was a specimen of his *Eimeria oxyspora*. A more thorough comparison, however, showed many differences:

1. The oöcysts are larger in my case (45 μ as compared with 36 μ).
2. The sporocysts are shorter (17–20 μ as compared with 30–32 μ).
3. The outer layer of the walls of oöcyst and sporocyst is rather indistinct.
4. The sporocystic residua are much more evident, and, on the contrary, an oöcystic residuum seems to be almost absent.
5. Dobell's "crystalline" bodies between the nucleus and the posterior end of the sporozoites are absent.

As for the cysts of *Eimeria wenyoni*, they also have a certain resemblance with the cysts here described; *e.g.* absence of oöcystic residuum, absence of the "crystalline" bodies, presence of highly refractile sporocystic residua, relative smoothness of the outer surface of the sporocystic wall. But undoubtedly the differences are more striking than the resemblances. Thus, in the first place, the cysts here described are more than twice as great in diameter (45 μ as compared with 20 μ); secondly, the spores are more slender, being of the same breadth but nearly twice as long as those of *Eimeria wenyoni*; thirdly, as a general rule, the ends of the sporocysts are acute and not obtuse; finally, the sporocystic residua are of a smaller size.

Moreover, it may be noted that I found in my case some undifferentiated and incompletely differentiated oöcysts in the stools, in contradistinction to the facts known of *Eimeria wenyoni* and *E. oxyspora*. But, with our present knowledge of this matter still being rather fragmentary, it is difficult to estimate the exact value of this difference.

Concerning the other coccidial parasites in man (hepatic *Eimeria* and *Isospora hominis*) I think confusion is impossible.

For all these reasons we are forced to accept the conclusion that the cysts in question belong to a species *hitherto undescribed in human stools*.

II.

Now if we find unexpected things in the stools we always have to reckon with three possibilities, and I think it a great mistake to omit consideration of any of them:

A. *The stool is contaminated after its deposition.* In our case I think this impossible, because the stool was passed into a dry, clean Petri dish; it was examined only half-an-hour later; and, above all, because we found the oöcysts thoroughly mixed up with the faecal mass and not only at its surface.

> I have some reason for laying stress on this point, because I saw a case of a Javanese child wrongly diagnosed at the first examination as a case of Balantidiosis. This child was suffering from a severe attack of dysentery with bloody stools and high fever. In the stools the laboratory assistant found numerous protozoa, resembling *Balantidium*, many of which contained red blood-corpuscles, this being supposed an incontestable sign of pathogenicity. So it was proclaimed as a case of Balantidiosis.

When I saw the preparations it struck me that there were also a lot of small protozoa, probably of quite different species. Now it turned out that the pots used to be washed in a little river. Consequently, nearly each pot contained a small quantity of water abounding with protozoa, most of which belonged to the genus *Paramecium*. After adding to the preparation under the microscope a drop of a suspension of erythrocytes one saw that the peristomal cilia of the *Paramecia* caused a stream, by means of which the red corpuscles—amongst other small particles—were engulfed in the mouth.

A bacteriological examination of another motion of the child passed afterwards into a sterile Petri dish showed the bacilli of Shiga-Kruse.

B. *The cysts are ingested with food or water and pass unaltered* (or only slightly altered) *through the alimentary canal.* Thus they appear in the stools where eventually they may continue their development.

Such is, for example, the case with *Bodo* ("*Prowazekia*") cysts. On the East Coast I can get "*Prowazekia*" only out of the faeces of people who drink unfiltered water.

In the case here described I think this supposition rather speculative, though the fact that a certain number of cysts proved to be dead, might seem to support it. We would have then to accept, in the *first* place, a thorough pollution of water or certain food-stuffs with animal faeces (or possibly with other animal excretions), *e.g.* the faeces of aquatic animals, or of cockroaches, flies, ants, mice, or rats; in the *second* place, an abnormally good preservation of the great majority of the cysts on their passage through the alimentary canal of Mr C., *i.e.* a very remarkable resistance of the wall of the ripe oöcysts to the digestive action of the human gastric and pancreatic juices.

That these two conditions, however, should both be satisfied is rather improbable, especially as I have never met with similar cysts in the stools or intestines of the above-mentioned animals. Neither have I been able to find any reference, in the literature at my disposal[1], to an identical form in animals. The oöcysts of all other species are smaller, at least as far as the descriptions are clear enough to rely on and the dimensions given are exact.

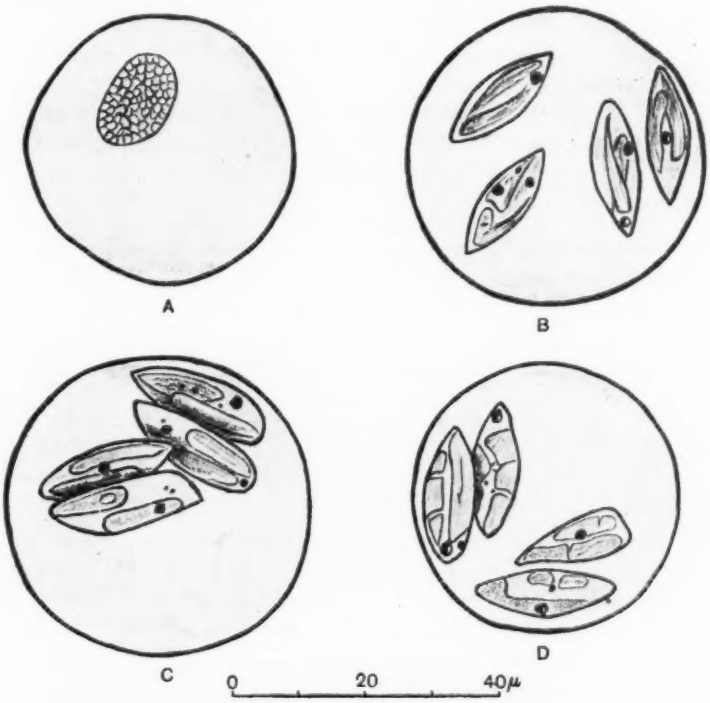
As to the second condition; of course it is not impossible that the wall of the oöcyst should be soluble in the digestive juices of the specific host only. This would represent then a high degree of physiological adaptation. But the facts known about the eggs of entozoa and the cysts of amoebae do not indicate the existence of such subtle differences in this respect.

But so far as I am aware we cannot deny this possibility (*water or food contamination*) altogether; in any case it is prudent to bear it in mind, and in certain cases it may prove to be worth a thorough investigation.

C. *The* Eimeria *is really parasitic in the described case, e.g.* as a parasite of the epithelium of the small bowel. (This last suggestion is probable because of the absence of liver-symptoms and because of the behaviour of *Eimeria* in

[1] The common Handbooks; *Arch. f. Protistenkunde*; *Centralbl. f. Bakt.*; *Parasitology.*

other animals.) If we accept this view, however, we must admit that its pathogenic power, if present, is of little importance, and either that the formation of an appreciable number of cysts very seldom occurs, *or that the infection is most transitory.*



The figures were drawn from the oöcysts as they appeared in a suspension of the fresh faeces in a 2 per cent. solution of eosin (with 0·9 per cent. NaCl). They are necessarily somewhat schematic. [The author's original figures (D excepted) show a faint line externally which delimits the cyst wall; this line unfortunately has been omitted from the figure as reproduced. The fine outer line should circumscribe the cyst at a distance equal to the thickness of the black contour.—ED.] Magnification × 1000.

A. Oöcyst with undifferentiated sphere showing a honeycomb structure.
B. Completely differentiated oöcyst, containing typical sporocysts and sporozoites.
C. The same, but sporocysts and sporozoites somewhat irregular.
D. Oöcyst with atypical sporozoites, looking "fixed," coagulated, and staining red with eosin.

If we now compare my case with those of Wenyon, Roche, and Dobell, we see that they are similar in the above-mentioned respects; and it should be noted that in most cases of *Isospora* infection also this absence of clinical symptoms and this short duration are circumstances almost constantly present.

So this sudden incidence and abrupt termination of the infection (or, at least, of cyst production), *and the absence of clinical symptoms seem to be peculiar properties of the intestinal coccidial infections in man,* and are certainly no obstacle to the acceptance of our third supposition.

Thus it may be concluded (though I must admit the impossibility of proving it to the hilt) *that the explanation which lies nearest to truth is that this case is one of parasitization of man by a hitherto undescribed* Eimeria, *which is probably peculiar to this host.*

The solution of the difficulties which we always meet with in rare infections, viz. the sources of infection, the modes of transmission, and the ways by which the infecting species preserves its continuity, must be reserved for further investigations.

MEDAN, *July* 1920.

REFERENCE.

DOBELL, C. (1919). A revision of the Coccidia parasitic in Man. *Parasitology,* XI. 147.

A NOTE ON THE NEW SPECIES OF *EIMERIA* FOUND IN MAN BY DR E. P. SNIJDERS.

By CLIFFORD DOBELL, F.R.S.

In the foregoing paper Dr E. P. Snijders has described the oöcysts of a species of *Eimeria* which he found in the stools of a patient under his care in Medan (Sumatra). The manuscript of this paper, together with a preparation containing the cysts in question, was kindly sent to me by the author[1] with the request that I would "be so kind as to add my opinion, and give to the species its right name." I shall do my best to comply with both these requests in the present note.

Dr Snijders has so fully described the cysts which he found, and has discussed their nature so ably, that there is little for me to add to his account. Careful examination of the specimen which he has sent me has suggested, however, a few points which appear worthy of note.

The specimen was a wet smear preparation of faeces, stained with iron haematoxylin and eosin and mounted in balsam. Dr Snijders describes it as "a bad one," but says he "could get no better." Nobody who has ever tried to make satisfactory permanent preparations of coccidial oöcysts and spores will be disposed to find fault with him on this score. It is frequently impossible to cause either fixatives or stains to penetrate these very resistant structures, and at present they can, in many cases, be studied properly in the fresh state only. I have never been able to obtain satisfactory stained preparations of any of the other coccidia of man.

On examining the preparation in question I was unable to recognize any oöcysts in it with certainty. I found, here and there, a few structures which appeared to be degenerate sporozoites, stained pink with eosin; but I was unable to make out the sporocysts or oöcysts enclosing them. I knew from experience, however, that the cysts of such organisms are often invisible when mounted in balsam—the cyst-walls and the balsam having approximately the same refractive index. I therefore removed the balsam with xylol, detached the cover-glass, and after passing the preparation through the various grades of alcohol, remounted the film in water. On re-examining it with a good lens, and suitably adjusted illumination, I was gratified to find a number of easily recognizable oöcysts—many of them, unfortunately, collapsed—containing

[1] I received the communication through the Editor of *Parasitology*: and I have therefore to thank Professor Nuttall not only for his good offices in this connexion but also for enabling me to publish this note simultaneously with Dr Snijders' paper.

spores whose walls (sporocysts) were quite intact. The pink-stained sporozoites within them appeared very degenerate, but were still recognizable. I made a number of careful measurements of the oöcysts and spores as they appeared in water. I then attempted to restain the preparation—with little success—and have remounted it permanently in euparal. In this medium, which has a lower refractive index than balsam, the cyst walls are barely visible.

I have had little difficulty in confirming Dr Snijders' statement that the cysts are those of a species of *Eimeria*. Every oöcyst contains the typical four spores, each of which—in all specimens sufficiently well preserved for study—encloses two sporozoites. Dr Snijders states that the diameter of the oöcysts is 40–48 μ. Most of those which I have seen are now too shrivelled for it to be possible to measure them accurately; but the few measurements which I have been able to make, and my estimates of the probable dimensions of the collapsed cysts, agree with his observations.

As regards the spores—which, as already noted, were still intact in many instances—I would note the following points. Dr Snijders states that they are whetstone-shaped, and measure 17–20 μ in length, with a breadth of 7–8 μ. They are shown best, I think, in his Fig. B: the more irregular outlines of Figs. C and D being due, apparently, to the fact that the spores in these were not all lying flat, but were seen more or less obliquely inclined to the axis of the microscope. It seems to me probable that Dr Snijders has not made sufficient allowance for this obliquity of many of the spores in making his measurements. At all events all my own measurements—made with great care from spores lying at right angles to the optical axis of the microscope, or estimated from those lying inclined to it at various angles—show that the length of the spores is slightly greater than he states. All my measurements lie between 20 μ and 25 μ, most of the spores measuring 22–24 μ. I find the width at the middle is—as stated by Dr Snijders—approximately 7–8 μ. (This dimension is not, of course, subject to the same error in its determination as the length.) All the spores appear to be fusiform, with their two ends equally pointed. Traces of an epispore ("mucous outer layer" of Dr Snijders) are visible on some; and the remains of sporocystic and oöcystic residual bodies can be made out in the majority of the cysts. It was impossible, however, to make out any details in the sporozoites.

It seems to me probable that the unsegmented oöcysts referred to by Dr Snijders, and shown in his Fig. A, are abnormal specimens. The protoplasmic inclusion appears far too small for a normal form in an early stage of development; but such abnormal stages are often seen in other species, and represent, I believe, dead oöcysts which have failed to get fertilized.

The oöcysts of this species are of unusually large size, and are certainly the largest described from man; but they are not the largest known in the Coccidia. Those of *Aggregata*, for example, attain much greater dimensions.

Although I was struck, at first, by the resemblance of these oöcysts to

those of *Eimeria oxyspora*, it seems clear, from their dimensions and those of their spores, that they belong to a distinct species. The dimensions of the spores in the Coccidia are very constant; and I do not know of any accurately described species which shows so great a range of variation in the form and size of its spores as that between *E. oxyspora* and the present organism. The relatively short spindle-shaped spores of the latter are quite distinct from the long whetstone-shaped spores of the former.

In my "Revision of the Coccidia parasitic in Man" (1919) I have discussed all the species of this group previously described: and Dr Snijders has pointed out that his *Eimeria* does not appear to belong to either of the intestinal species of this genus therein recognized—*E. wenyoni* and *E. oxyspora*. In this I fully agree; and I think Dr Snijders is to be congratulated, therefore, upon having demonstrated the existence of a hitherto undiscovered coccidium in man. There is a possibility, as he rightly points out, that the oöcysts may be those of an *Eimeria* belonging to some other host; but there is, I believe, no evidence at present in favour of such a supposition. It seems probable that the parasite is one which belongs to man himself. It is, however, somewhat remarkable that all the species of *Eimeria* hitherto found in human stools are not only very rare, but apparently cause infections which are peculiarly transitory. Their cysts have suddenly appeared in the stools and then promptly vanished—never to return.

Since I wrote my revision of the Coccidia of man, two papers purporting to describe new human cases of intestinal coccidiosis have appeared. The first, by Huetter (1919), records the finding of "coccidia" in sections of a rectal tumour from a woman: but even from the incomplete description of this case, it seems obvious that it was not really one of coccidiosis. Of the other case of "coccidiosis (?)" recorded by Lockhart-Mummery and Gabriel (1919) I can speak with more confidence. I have seen a preparation of the structures interpreted as coccidia, and have no hesitation in saying that they are neither coccidia nor protozoa of any sort.

Brumpt (1918) has recently stated that the French Armies were infected to the extent of 0·2 to 0·33 per cent. with "*Eimeria* (*Coccidium*)": but—so far as I am aware—he has not described this organism. Chatton (1918, p. 218) states that he has found three cases of *Eimeria* infection in Southern Tunis, but does not name or describe the species. According to Mesnil (1919) it was probably *E. wenyoni*.

So far as I am aware, therefore, Dr Snijders' *Eimeria* belongs to a species which has not hitherto been described or named. I propose to call it *E. snijdersi*, in honour of its discoverer, and give the following diagnosis. For reasons already stated I have ventured to change the dimensions of the spores, as given by Dr Snijders, to those which I believe, from my own measurements of some of his specimens, to be correct.

Eimeria snijdersi nov. spec.

Oöcyst colourless, spherical, 40–48 μ in diameter. Spores fusiform, equally

pointed at both ends; length 20–25 μ, width in middle 7–8 μ. Oöcystic residue small, granular. Sporocystic residues in the form of one or two small refractile spheres. No "crystalline bodies"—like those of *E. oxyspora*—visible at the posterior ends of the sporozoites.

Habitat: intestine (?) of man.

As yet found in one case only, at Medan (Sumatra).

The structural characters given above serve to distinguish this species readily from the two other species of *Eimeria* previously described from human stools—*E. wenyoni* and *E. oxyspora*[1].

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REFERENCES.

Brumpt, E. (1918). Protozoaires et Helminthes des selles aux Armées. *C. R. Soc. Biol.* lxxxi. 1044.

Chatton, E. (1918). Le Laboratoire militaire de Bactériologie du Sud-Tunisien (à Gabès), etc. I. Flore et faune intestinales. *Arch. Inst. Pasteur, Tunis*, x. 205.

Dobell, C. (1919). A Revision of the Coccidia parasitic in Man. *Parasitol.* xi. 147.

Huetter (1919). Menschliche Darmcoccidiose. *München. med. Wchnschr.* lxvi. 730.

Lockhart-Mummery, P., and Gabriel, W. B. (1919). Case of intestinal coccidiosis (?). *Proc. Roy. Soc. Med.* xiii. (Sect. Surg., Subsect. Proctol.), 14.

Mesnil, F. (1919). [Review of *Dobell* (1919).] *Bull. Inst. Pasteur*, xvii. 376.

[1] I may note that M. Mesnil (1919) has, by an unfortunate misprint, inadvertently renamed this species "*E. oxyphila*."

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